www.czasopisma.pan.pl PAN www.journals.pan.pl
POLSKA AKADEMIA NAUK

Polish Journal of Veterinary Sciences Vol. 15, No. 3 (2012), 547-552

DOI 10.2478/v10181-012-0084-5

Original article

Effects of Se deficiency on serum histamine concentration and the expression of histamine H_2 receptor in the jejunum of chickens

Guo-qing Wang[1], Hong-hai Wang[2], Hai-xia Wang[3]

[1] College of Sciences, Northeast University, 3 Wenhua Road, Shenyang, 110004, China
[2] Animal Husbandry Bureau of Daqing city, 28 Yucai Road, Daqing, 163311, China
[3] College of Animal Science and Veterinary Medicine,
Jilin University, 5333 Xi'an Road, Changchun, 130062, China

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 H_2 receptor (H_2R) 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 H_2R 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 H_2R promotes both regulation of digestion and cell proliferation while protects the jejunum from injury induced by Se deficiency.

Key words: selenium deficiency, histamine, histamine H_2 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 defensing 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

Correspondence to: GQ Wang, e-mail: wgq20082003@yahoo.com.cn

(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 H_2 receptor (H_2R) 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 Na_2SeO_3 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 Stafilov 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) 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.

Gene	Primer sequence	Fragment size (bp)
H_2R EF193862	Forward 5'-GCACTGAACCCCATCCTCTA-3' Reverse 5'-CTTCCCATTCCTCATCTCCA-3'	192
β-actin L08165	Forward 5'-ACCGCAAATGCTTCTAAACC-3' Reverse 5'-CCAATCTCGTCTTGTTTTATGC-3'	93

subjected to reverse transcription to form cDNA with transcriptase according to the manufacturer's instructions (Invitrogen, USA). The mRNA expression of the H_2R 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.

Age in days	Se-deficient group (µg/mL)	Control group (µg/mL)
30	0.199 ± 0.031^{Ab}	0.267 ± 0.019^{Aa}
45	0.153 ± 0.020^{BAc}	0.248 ± 0.018^{Aa}
60	0.093 ± 0.022^{Dd}	0.255 ± 0.054^{Aa}
75	0.049 ± 0.012^{De}	0.287 ± 0.036^{Aa}

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.

Age in days	Se-deficient group (ng/g)	Control group (ng/g)
30	5.950 ± 0.569^{Dd}	3.329 ± 0.257^{Ef}
45	6.792 ± 0.392^{Cc}	3.886 ± 0.172^{Eef}
60	10.358 ± 0.911^{Bb}	4.066 ± 0.185^{Ee}
75	11.229 ± 0.592^{Aa}	3.865 ± 0.163^{Eef}

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 H_2R mRNA expression

The level of H_2R mRNA expression in the Se-deficient group was significantly higher than that in the control group (Fig. 3). The difference between the

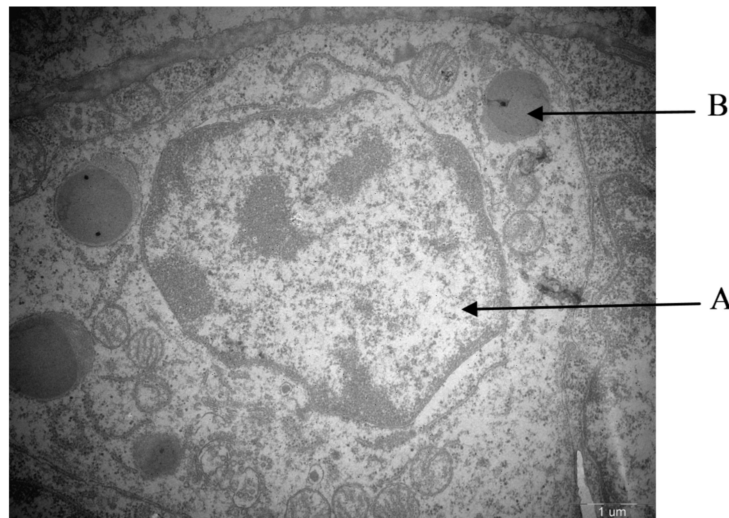

Fig. 1. MC ultrastructure in the control group (×25000). Differently sized, homogeneously distributed, round/oval granule were found in the MC. A. MC nucleus, B. MC grains.

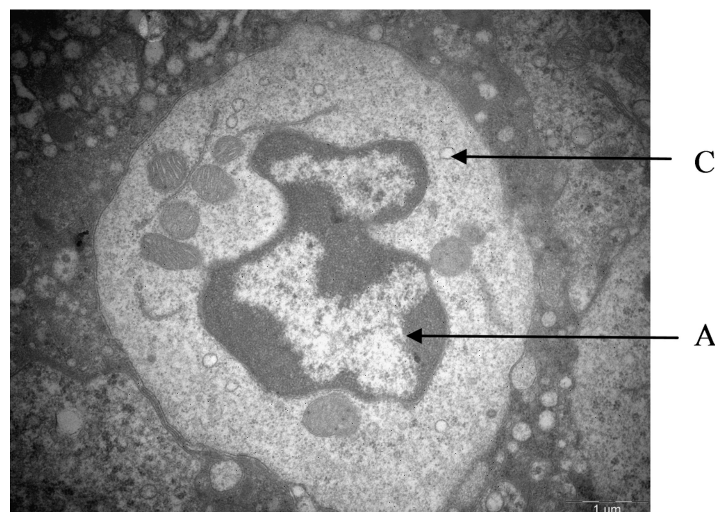

Fig. 2. MC ultrastructure in Se-deficient group (×25000). MC showed an excess of heterochromatin, chromatin condensation, larger grains, and denser cytoplasm and vacuoles. A. MC nucleus, C. MC grains vacuole.

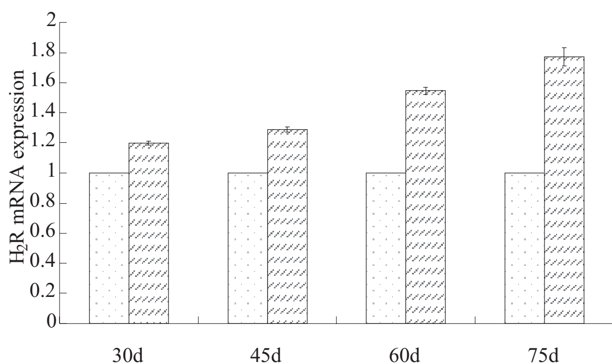

Fig. 3. H_2R mRNA expression. The H_2R mRNA expression level in the Se-deficient group was significantly higher than that in the control group (P<0.01).

control group and the Se-deficient group was highly significant (P<0.01) and exhibited a time effect.

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).

H_2R is coupled positively to adenylyl cyclase. Activation of these receptors also leads to mainly excitatory effects through blockade of Ca^{2+}-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 Ca^{2+}-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 H_2R. Histamine released by MC is paracrine mitogen, the effect of which is mediated by H_2R (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), Ca^{2+} signal transducting system. H_2 promotes cell proliferation referred to c-fos genetic transcription (Shayo et al. 1997, Wang et al. 1997). Therefore, histamine and H_2R play a regulatory role in maintaining function of intestines and promoting enterocyte proliferation. Se deficiency increases jejunal H_2R mRNA expression level, indicating that H_2R protects the jejunum, thus avoiding injury during Se deficiency.

Acknowledgments

This project was supported by the Research Foundation from the Ministry of Education of China and Northeastern University (20080012, 20060603).

References

Akdis CA, Simons FE (**2006**) Histamine receptors are hot in immunopharmacology. Eur J Phamracol 533: 69-76.

Berin MC, Kiliaan AJ, Yang PC, Groot JA, Kitamura Y, Perdue MH (**1998**) The influence of mast cells on pathways of transepithelial antigen transport in rat intestine. J Immunol 161: 2561-2566.

Bozkaya LA, Oztürk-Urek R, Aydemir T, Tarhan L (**2001**) Effects of Se, Cu and Se^+ vitamin E deficiency on the activities of Cu, Zn, SOD, GSH-Px, CAT and LPO levels in chicken erythrocytes. Cell Biochem Funct 19: 153-157.

Brown RE, Stevens DR, Haas HL (**2001**) The physiology of brain histamine. Prog Neurobiol 63: 637-672.

Church MK, Clough GF (**1999**) Human skin mast cell: in vitro and in vivo studies. Ann Allergy Asthma Immunol 83: 471-475.

Church MK, Levi-Schaffer F (**1997**) The human mast cell. J Allergy Clin Immunol 99: 155-160.

Delvalle J, Wang L, Gantz I, Yamada T (**1992**) Characterization of H_2 histamine receptor: linkage to both adenylate cyclase and $[Ca^{2+}]i$ signaling systems. Am J Physiol 263: G967-G972.

Entschladen F, Drell TL, Lang K, Joseph J, Zaenker KS (**2004**) Tumour-cell migration, invasion, and metastasis: navigation by neurotransmitters. Lancet Oncol 5: 254--258.

Franzen L, Norrby K (**1980**) Local mitogenic effect of tissue mast cell secretion. Cell Tissue Kinet 13: 635-642.

Fujitani T, Adachi N, Nagaro T, Miyazaki H, Nakamura Y, Kataoka K, Arai T (**1996**) Histaminergic H_2 action protects hippocampal CA1 neurons by prolonging the onset of the anoxic depolarization in gerbils. J Neurochem 67: 2613-2615.

Gushchin IS, Petyaev IM, Tsinkalovsky OR (**1990**) Kinetics of oxygen metabolism indices in the course of histamine secretion from rat mast cells. Agents Actions, 30: 85-88.

Hill SJ (**1990**) Distribution, properties, and functional characteristics of three classes of histamine receptor. Pharmacol Rev 42: 45-83.

Katzka DA (**2000**) Digestive system disorders, gastroesophageal reflux disease. West J Med 173: 48-53.

Lazaru A, Stafilov T (**1998**) Determination of copper in sulfide minerals by Zeeman electrothermal atomic absorption spectrometry. Fresen J Anal Chem 360: 726-728.

Metcalfe DD, Baram D, Mekori YA (**1997**) Mast cells. Physiol Rev, 77: 1033-1079.

Mitsuhashi M, Mitsuhashi T, Payan DG (**1989**) Multiple signaling pathways of histamine H_2 receptors. Identification of an H_2 receptor-dependent Ca^{2+} mobilization pathway in human HL-60 promyelocytic leukemia cells. J BioI Chem 264: 18356-18362.

Muller AS, Pallauf J, Most E (**2002**) Parameters of dietary selenium and vitamin E deficiency in growing rabbits. J Trace Elem Med Biol 16: 47-55.

Nishi Y, Takesue Y (**1978**) Electron microscope studies on triton-solubilized sucrase from rabbit small intestine. J Ultrastruct Res 62: 1-12.

Ohta Y, Kobayashi T, Nishida K, Ishiguro I (**1997**) Relationship between changes of active oxygen metabolism and blood flow and formation, progression, and recovery of

lesions in gastric mucosa of rats with a single treatment of compound 48/80, a mast cell degranulator. Dig Dis Sci 42: 1221-1232.

Pfaffl MW, Gerstmayer B, Bosio A, Windisch W (**2003**) Effect of zinc deficiency on the mRNA expression pattern in liver and jejunum of adult rats: monitoring gene expression using cDNA microarrays combined with real-time RT-PCR. J Nutr Biochem 14: 691-702.

Saito Y, Yoshida Y, Akazawa T, Takahashi K, Niki E (**2003**) Cell death caused by selenium deficiency and protective effect of antioxidants. J Biol Chem 278: 39428-39434.

Santos J, Yang PC, Soderholm JD, Benjamin M, Perdue MH (**2001**) Role of mast cells in chronic stress induced colonic epithelial barrier dysfunction in the rat. Gut 48: 630-636.

Shayo C, Davio C, Brodsky A, Mladovan AG, Legnazzi BL, Rivera E, Baldi A (**1997**) Histamine modulates the expression of c-fos through cyclic AMP production via the H_2 receptor in the human promonocytic cell line U937. Mol Pharmacol 51: 983-990.

Wang LD, Hoeltzel M, Butler K, Hare B, Todisco A, Wang M, Del Valle J (**1997**) Activation of the human histamine H_2 receptor is linked to cell proliferation and c-fos gene transcription. Am J Physiol 273: C2037-C2045.

Wang J, Li SH, Liang L, Jiang QF, Zhou JX, Ye FY, Huang L (**2009**) Effect of Se-enriched yeast cultured in Chinese medicine on microstructure of the liver and small intestine of Minzhongma chickens. Chinese Vet Sci 39: 1114--1119.

Wang ZX, She RP, Chen Y, Chen YX, Cheng AW (**2003**) Effect of different quantities of zinc and selenium level in diet on the structure of mucous epithelium and intraepithelial lymphocyte and the goblet cells in the small intestine of broiler. Sci Tech Engi 3: 568-572.

Yang PC, Berin MC, Yu L, Perdue MH (**2001**) Mucosal pathophysiology and inflammatory changes in the late phase of the intestinal allergic reaction in the rat. Am J Pathol 158: 681-690.

Zhu Z, Kimura M, Itokawa Y, Aoki T, Takahashi JA, Nakatsu S, Oda Y, Kikuchi H (**1996**) Apoptosis induced by selenium in human glioma cell lines. Biol Trace Elem Res 54: 123-134.