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Copper and arsenic-induced oxidative stress and immune imbalance are associated with activation of heat shock proteins in chicken intestines

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ABSTRACT

Arsenic and copper, two ubiquitous pollutants, can be oxidative stress inducers when organisms are heavy or chronically exposed, causing adverse effects on digestion and absorption function, resulting in potential losses to poultry husbandry. The present study examined the effects of arsenic trioxide (30 mg/kg)- and copper sulfate (300 mg/kg)-mixed foods, administered alone or in combination for 12 weeks, on various biochemical indices of oxidative stress and immunity in the small intestines of Hy-line chickens. The results showed that for the first four weeks of exposure, both the redox and immune systems were unaffected. Subsequently, exposure to arsenic or copper significantly increased the level of lipid peroxidation (malondialdehyde and ability of anti-hydroxy radical) concomitant with a collapse of the antioxidant system (catalase and glutathione peroxidase), in a time-dependent manner. An increase in the mRNA and protein levels of pro-inflammatory indicators (nuclear factor kappa B, cyclooxygenases-2, tumor necrosis factor-α and prostaglandin E2 synthases) with a definite tendency toward Th1 (Th, helper T cell) cytokines was observed in both arsenic and copper treated chickens. Histologically, the destruction of the biofilm structure and inflammatory infiltrates was observed. Thus, in the intestine, heat shock proteins play protective roles against tissue damage. In some cases, we observed that the tissues of the small intestine were more sensitive to arsenic than to copper. Moreover, co-exposure induced more serious intestinal toxicity than single treatment group, and this mechanism needs further exploration.

1. Introduction

As a well-known toxic pollutant, arsenic trioxide (As_2O_3) and its metabolites have disastrous and life-threatening consequences [1]. Currently, the spread of arsenic has been exacerbated by increased human activities including mineral mining and fuel combustion, ultimately entering the groundwater through geothermal, hydrological, and biochemical processes [2]. However, copper (Cu) is an essential micronutrient in living organisms and contributes to optimal cellular function. Copper sulfate, the compound used to induce oxidative stress (OxS) in the present study, is a common ingredient in multivitamins [3]. However, this compound features a narrow optimal range between essential and toxic concentrations. In addition, many copper (Cu)-based pesticides, including cupric sulfate, copper oxychloride and cupric carbonate, are extensively used worldwide. These agrochemicals exert their toxic effects by metabolizing uniquely in different living organisms [4]. Several lines of evidence suggest that these inorganic pollutants are responsible for many hematological, immune and digestive disorders, which mostly have a bearing on oxidative damage [5]. Thus, areas cross-contaminated with copper and arsenite are frequently encountered.

The deleterious effects of copper and arsenite on the biological system are largely caused by the formation of ROS, comprising hydrogen peroxide (H_2O_2), superoxide anion ($O_2\cdot^-$) and hydroxyl radical ($\cdot OH$), which overwhelm the ability of an organisms to counteract OxS [6]. These ROS may generate OxS that may adversely affect macromolecules [7]. OxS was reported to be related to arsenicosis in the spleen, thymus and Bursa of Fabricius [8] and brain tissues [9] of chickens. In addition, some studies have shown that copper-induced OxS is clearly dependent on the Cu^{2+} concentration, characterized by the origination of lipid peroxidation (LPO) and a collapsed antioxidant enzyme system [10,11].

OxS is as an essential component of the signaling cascade of inflammatory bowel diseases (IBD) [12], a stress response characterized by mucosal disruption and ulceration. ROS also function as second messengers in several signal transduction pathways including nuclear factor-κB (NF-κB) signaling, whose signaling cascade plays a crucial role in the initiation and amplification of the inflammation via the

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modulation of multiple inflammatory mediators [13]. However, recent studies have suggested that ROS regulate the activity of NF-κB in a bidirectional manner [14]. Previous studies have shown a tight relationship between NF-κB and some inflammatory mediators, including prostaglandin E2 synthases (PTGEs), cyclooxygenases-2 (COX-2) and tumor necrosis factor-alpha (TNF-α) [8,15]. NF-κB also participates in T-cell functional divergence and development, such as the differentiation of Th 1 and Th2 (helper T cell) [16]. The invasion of the intestinal tissue by xenobiotics may also trigger a well-coordinated and tightly regulated immune response involving a number of different leukocytes including T cells [17]. These effector T cells then return to systemic circulation, and home to the gut lamina propria where the pathogen is located. Th1- and Th17-derived inflammatory cytokines (e.g., interferon gamma (IFN-γ), interlukin-17 (IL-17), and TNF-α) interact with and activate antigen-presenting cells and tissue macrophages resulting in the generation of additional inflammatory mediators including IL-1β, IL-2, IL-6, IL-8, and IL-12 as well as various ROS (e.g., $O_2\cdot^-$, ·OH, H_2O_2) [18]. In contrast, therapeutic strategies to induce antigen-specific Th2 responses, are accompanied by the secretion of IL-4 and IL-10. Hence, the imbalance of antigen-specific pro-inflammatory and anti-inflammatory cytokine responses is a key to drive the development and progression of IBD.

Heat shock proteins (HSPs) are molecular chaperones that are conservatively expressed in all living organisms. These proteins can be up-regulated by a variety of physiological, emergency and environmental insults. Such proteins interact with antigen-presenting cells and are capable of stimulating strong Th1 responses when utilized as adjuvants [19]. HSP27 effectively scavenges ROS to improve the balance of the oxidation-reduction system, thereby reducing the damage to human colonic epithelial cells [20]. In gut epithelial cell lines, the positive impact of HSP27 to mitigate OxS has been adequately demonstrated [21]. However, in another ongoing stress environment, in which improved HSP60 expression modulated the cellular anti-stress response, mitochondrial dysfunction subsequently mediated OxS, which in turn culminated into the sequestered efficacy of the immune system and increased mortality [22]. Thus, when the cell or organism succumbs to a non-fatal stress response, the over-expression of HSPs may confer protection from injury.

The chicken small intestine comprises three sections-, namely, the duodenum, jejunum and ileum-, each with varying functions related to nutrient digestion, absorption and shielding harmful substances. As an independent immune system and, the main part of the mucosal immune system (MIS), intestinal mucosal immunity provides the first line of defense of the inner body surface of the adaptive anti-inflammatory response [23]. Secretory Immunoglobulin A (sIgA) is an effective dominant factor in the MIS that functions in antigen neutralization and opsonization, thus reflecting immune state of the body [24]. Literature analysis also shows that many pro-inflammatory and immune-regulatory cytokines, including IL-4, IL-6, IL-10, TNF-α and IFN-γ, have a mobilizing effect on the differentiation of B cells and the synthesis of IgA antibodies [25,26]. However, under the influence of endogenous or exogenous stressors, excessive free radicals are produced, which induce OxS and intestinal mucosal damage in the digestive tract [27]. This damage can have adverse effects on animal growth and development and bring serious losses to poultry husbandry. To date, few studies have reported the effects of copper and arsenic co-exposure on chicken small intestinal tissues. Therefore, given the threat of environmental pollutants, the present study assessed the intestinal toxicity of the individual or joint exposure of $CuSO_4$ and As_2O_3 with respect to oxidative and immune imbalance. The present study will provide insight into the beneficial role of HSPs in this context.

2. Materials and methods

2.1. Animals and treatment

The Animal Care, Use and Ethics Committee of Northeast Forestry University (approval no. UT-31; 20 June 2014) is responsible for the review and approval of this animal experiment. Chickens (72 individuals; Hy-line strain; male; 1-day-old; ~50 g; Weiwei Co. Ltd., Harbin, China) were housed in the Institutional Animal Care of Northeast Forestry University. Consistent the reasonable dose of subchronic toxicity test (1/20 to 1/5 of the median lethal dose (LD50)) [Supplementary Appendix. 1], and the LD50 of arsenic trioxide for chickens (50 mg/kg BW) [Supplementary Appendix. 2], As_2O_3 (2.5 mg/kg BW, corresponding 30 mg/kg feed) was added supplemented into the food. However, although the addition of 125–250 mg/kg copper of feed results in gaining in increased feed efficiency and body weight (BW) [28], a $CuSO_4$ concentration of 250 mg/kg in the diet resulted in reduced feed intake [29] and that of 300 mg/kg causes growth depression in chickens [28]. The above studies determined the dose of copper in the present study (300 mg/kg feed). Chickens were divided at random into 4 groups (18 individuals per group), containing a control group: standard diet, and three experimental groups: copper-group, arsenic-group or co-exposure group. The feed ingredients (Table 1) and rearing conditions of experimental animals were used according to a previous study [8]. Six individuals in each group were randomly sacrificed at the 4th, 8th and 12th weeks and euthanized with sodium pentobarbital (30 mg/kg BW). The small intestinal tissues (duodenum, jejunum and ileum) were quickly excised and blotted then rinsed with ice-cold 0.9% NaCl solution, immediately frozen in liquid nitrogen, and stored at −80 °C until further analysis.

2.2. Histological and ultrastructural observations

The intestinal tissues specimens (1.0 mm^3) were rapidly extracted from all four groups at the 12th weeks. Hematoxylin and eosin (H&E) staining was used in histological analysis. After the ultrathin section, uranyl acetate and citromalic acid lead double staining. A JEM-1200ES electron microscope was used in ultrastructural observation.

2.3. Determination of the antioxidant system

The intestinal tissues sampled at different time-points were collected to determine the oxidation state (n = 6/group). The OxS indices indicated in the figures were detected by using detection kits (Jiancheng Bioengineering Institute, Nanjing, China) according to the

Table 1
Composition and nutrient content of the basal diet.

Ingredients	%	Nutrient content	%, unless noted
Corn	62.00	Metabolizable energy	MJ/kg 10.91
Soybean meal (44.20% CP)	22.00	Crude protein	15.51
Wheat bran	3.00	Lysine	0.78
Limestone	8.00	Methionine	0.34
Calcium phosphate	1.25	Tryptophane	0.16
Sodium chloride	0.40	Total phosphorus	0.48
Premix	3.35	Calcium	3.52
Total	100.00	Mercury, mg/kg	0.27

The premix provided following per kilogram of diet: vitamin A, 7000 IU; vitamin D3, 2500 IU; vitamin E, 49.5 mg; vitamin K3, 1 mg; vitamin B1, 1.5 mg; vitamin B2, 4 mg; vitamin B6, 2 mg; vitamin B12, 0.02 mg; niacin, 30 mg; folic acid, 0.55 mg; pantothenic acid, 10 mg; biotin, 0.16 mg; choline chloride, 400 mg; Cu, 5 mg; Fe, 70 mg; Mn, 100 mg; Zn, 70 mg; I, 0.4 mg; Se, 0.5 mg.