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Cell apoptosis of caprine spleen induced by toxicity of cadmium with different levels of molybdenum

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ABSTRACT

In order to clarify the effects of the combination of Mo and Cd on goat and relationship between the two elements, combined chronic toxicity of cadmium with different levels of molybdenum *in vivo* on apoptosis gene and ultrastructure of spleen was evaluated with the methods of RT-qPCR and transmission electron microscopy. A total of thirty-six goats were randomly distributed in equal number into four groups. These groups were randomly assigned with one of three oral treatments of $CdCl_2$ (0.5 mgCd kg^{-1}) and $[(NH_4)_6Mo_7O_{24}{\cdot}4H_2O]$ (15 mg Mo kg^{-1}, group I; 30 mg Mo kg^{-1}, group II; 45 mg Mo kg^{-1}, group III), while the control group received deionized water. Spleen tissues were taken from individual goat at different time intervals to measure the levels of apoptosis genes including *Bcl-2*, *Bax*, *Cyt c*, *Caspase-3*, *Smac* and ceruloplasmin (*Cp*). The results revealed that a significant suppression in *Bcl-2* expression and increase in *Cyt c*, *Caspase-3* and *Cp* expression in splenic cells. The *Bax* expression in group I and II was up-regulated, however, it displayed reduction in group III, whereas no statistical significance was observed on *Smac* expression. In addition, histopathologic injury revealed remarkable morphplogical changes on the splenocytes in the means of apoptosis including fragmentized nucleus, apoptotic body and vesiculation of cytoplasma and mitochondria. Taken together, combined chronic toxicity of cadmium with different levels of molybdenum induce goat spleen cell apoptosis associated with mitochondrial intrinsic pathway, and the two elements showed possible antergic relationship.

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1. Introduction

Molybdenum (Mo) is as an essential trace element for many species since it can act as an indispensable component in xanthine oxidase (XOD), aldehyde (AO), sulfite oxidase (SO) (Richert and Westerfeld, 1953; Mahler et al., 1954; Cohen et al., 1971). Molybdenum is traditionally considered to be the cause of secondary Cu deficiency, furthermore, itself is toxic after ruminal transformation to thiomolybdates (Frank, 2004). Molybdenum toxicity has been reported in different species, such as mouse, rabbit, goat and sheep (Fairhall et al., 1945; Arrington and Davis, 1953; Dick, 1956; Hogan et al., 1971). In 1981, a suspect of Mo toxicity of cattle was initially

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investigated in China. It was "Red Skin and White Hair" syndrome described by local famers since it caused persistent diarrhoea, skin redness and hair depigmentation in cattle (Fan et al., 1981).

Cadmium (Cd) is a toxic metal emanated from the natural environment, and particularly due to industrial pollution. Cadmium is a non biodegradable metal and it has shown to accumulate continuously in the body and result in various disorders of the organs such as spleen and immune system (Ficek et al., 1994; Godowicz et al., 1988). Reports indicated that the cadmium stimulated the generation of large amounts of free radicals, resulting in biological macromolecules being under attack (Ognjanović et al., 2010), which may ultimately lead to apoptosis.

Mo might offer protection against the effects of increased heavy-metal concentrations (Rautio et al., 2010), such as Cd and Hg. It has been suggested that Na_2MoO_4 is capable of relieving the acute toxicity of $CdCl_2$ in rat, and the protective mechanism of the metal is partly associated with the enhancement of liver Cd–metallothionein induction (Yamane et al., 1990). The accumulation of Cd in tissues of sheep decreased as dietary levels of Mo and sulfur (S) increased (Smith and White, 1997). The rates of Mo and Cd exposure in the Jiangxi Province of China are much higher than other territories due to superfluous industries and over developed mining (Dai et al., 1993), and animals have high probability to encounter mixed toxicosis due to abundant mineral resources. Therefore, this study has been conducted in order to discuss the effects of the combination of Mo and Cd on spleen of ruminant and discern the relationship between the two elements in goat. The methods of RT-qPCR and transmission electron microscopy have been applied to study toxic effects of molybdenum and cadmium on mRNA levels of apoptosis-related genes and ultrastructure changes in spleen *in vivo*.

2. Materials and methods

2.1. Animals and treatments

A total of 36 clinically healthy male Boer goats which were 20 kg to 30 kg in weighing approximately were procured from local farm, ageing between 1.5 years and 2 years, and were randomly assigned in equal number to 4 groups. The goats were acclimatized for two weeks in the college animal farm shed under hygienic conditions before the commencement of the experiment. Feed and water was provided *ad libitum* throughout the study. Goats in the control group were orally administered corresponding quantitative deionized water, while goats of the experiment groups were orally administered the same amount $CdCl_2$ (0.5 mg Cd kg^{-1} BW) with different levels of $[(NH_4)_6Mo_7O_{24}{\cdot}4H_2O]$ (15 mg Mo kg^{-1}, 30 mg Mo kg^{-1}, 45 mg Mo kg^{-1}) according to their body weight. In this case, groups consisted of one control group in one side, and experiment groups (i.e. group I, group II and group III) in the other side. The daily administration of salts was made between 8.00 and 10.00 a.m. after dissolving them in adequate amount of deionized water, according to goats' body weights. All goats were closely observed for clinical signs and mortality. The time span for experiment persisted was 50 days. All animal care and experimental procedures were approved by the institutional ethic committee, and this study also complied with the criteria in Guide for the Care and Use of Laboratory Animals.

Table 1 – Composition and nutrient levels in the basal diet for the goats.

Composition of diet		Nutrient levels	
Ingredient	Content (%)	Index	Level
Maize	52.5	CP (%)	16.57
Deoiled rice bran	19.0	ME (MJ kg^{-1})	13.12
Soybean meal	10.0	Ca (%)	0.90
Rapeseed meal	7.0	P (%)	0.78
Cottonseed meal	7.0		
$CaHPO_4$	1.0		
Limestone	1.5		
Salt	1.0		
Additives*	1.0		
Total	100		

* Per kilogram of additives contained: nicotinic acid 2000 mg; V_A 1,000,000 IU; V_D 3,250,000 IU; V_E 2400 mg; Fe ($FeSO_4{\cdot}H_2O$) 2000 mg; Zn ($ZnSO_4{\cdot}H_2O$) 140,000 mg; Mn ($MnSO_4{\cdot}H_2O$) 3000 mg; I (KI, 3%) 180 mg; Se ($NaSe_3O_4{\cdot}H_2O$) 100 mg.

Table 2 – The content of Mo and Cd in water, grass, fodder μg g^{-1}.

Item	Micronutrient levels	
	Mo	Cd
Deionized water	0.0000	0.0000
Tap water	0.0089	0.0008
Grass	1.8888	0.0708
Fodder	6.0195	0.0496

2.2. Biohydrogenation

Basic biohydrogenation for goats were prepared according to the standard nutritional requirement for goat breeding by NRC (1981). The composition of basic biohydrogenation and the content of Mo and Cd in grass, water and fodder were shown in Tables 1 and 2. Molybdenum and cadmium analysis in grass, water and fodder were made using a Shimadzu AA 680 flame atomic absorption spectrophotometer (Shimadzu, Japan).

2.3. Sample collection

During sampling, spleen tissues were removed from twelve goats by random selection of three goats from each group, immediately after they were sacrificed with an overdose intravenous injection of sodium pentobarbital (Nembutal, Abbot Labs, IL, USA, 100 mg kg^{-1}) on days 0, 25 and 50.

2.4. Mo, Cd, Cu analysis

Mo, Cd and Cu levels of spleen were determined by a Shimadzu AA 680 flame atomic absorption spectrophotometer (Shimadzu, Japan) after wet-ashing the samples.

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