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Inhibition of peripubertal sheep mammary gland development by cysteamine through reducing progesterone and growth factor production

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ABSTRACT

Cysteamine has been used for treating cystinosis for many years, and furthermore it has also been used as a therapeutic agent for different diseases including Huntington's disease, Parkinson's disease (PD), nonalcoholic fatty liver disease, malaria, cancer, and others. Although cysteamine has many potential applications, its use may also be problematic. The effects of low doses of cysteamine on the reproductive system, especially the mammary glands are currently unknown. In the current investigation, low dose (10 mg/kg BW/day) of cysteamine did not affect sheep body weight gain or organ index of the liver, spleen, or heart; it did, however, increase the levels of blood lymphocytes, monocytes, and platelets. Most interestingly, it inhibited mammary gland development after 2 or 5 months of treatment by reducing the organ index and the number of mammary gland ducts. Plasma growth hormone and estradiol remained unchanged; however, plasma progesterone levels and the protein level of HSD3β1 in sheep ovaries were decreased by cysteamine. In addition to steroid hormones, growth factors produced in the mammary glands also play crucial roles in mammary gland development. Results showed that protein levels of HGF, GHR, and IGF1R were decreased after 5 months of cysteamine treatment. These findings together suggest that progesterone and local growth factors in mammary glands might be involved in cysteamine initiated inhibition of pubertal ovine mammary gland development. Furthermore, it may lead to a reduction in fertility. Therefore, cysteamine should be used with great caution until its actions have been further investigated and its limitations overcome.

1. Introduction

Cysteamine (β-mercaptoethylamine) is an essential portion of coenzyme A, which is highly conserved in mammals [1,2]. The plasma concentration of cysteamine in animals or humans is very low [3,4]. Initially, it was believed that functions involving cysteamine in mammals included the synthesis and oxidation of fatty acids, the oxidation of pyruvate in the citric acid cycle, and depletion of tissue somatostatin [5]. However, the exact mechanism of action for cysteamine is currently not understood. Cysteamine has one thiol group in its molecule, and the thiol bond can be oxidized into a disulfide bond, which in turn can be reduced back to a thiol bond. Therefore, cysteamine might have biphasic effects that suggestt a mechanistic action. Furthermore, cysteamine can act as an antioxidant to reduce oxidative stress in cells. At low concentrations, cysteamine can promote the transport of cysteine into cells; however, at higher doses and in the presence of transition metals, its oxidation can generate hydrogen peroxide (H2O2), which might result in oxidative stress [6].

Cysteamine has long been used as a treatment for different diseases. Initially, it was reported as a radio-protective agent in the 1950s [7]; later, in 1976, it was used to treat cystinosis and it is still the only treatment available for this disease. Cystinosis is characterized by cystine accumulation in cells throughout the body. It is

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a lysosomal storage disorder caused by mutations in the gene encoding cystinosin-lysosomal cystine transporter (CTNS) on chromosome 17p3. Patients with this disease have problems with generalized proximal tubular damage (called renal Fanconi syndrome), polyuria, polydipsia, and they fail to thrive during the first year of life. Cysteamine treatment decreases cysteine levels in cells and prolongs patient life. New applications for cysteamine include the treatment of Huntington's disease [8–10], Parkinson's disease (PD) [11,12], nonalcoholic fatty liver disease [13], malaria [14,15], cancer [16], sickle cell anemia [17], HIV-I [18], paracetamol (acetaminophen) hepatotoxicity [19], and as immunomodulatory agents [20].

Although cysteamine has been used to treat many diseases, it has also been used in livestock production [21–23]; however, its usage may cause problems. One common issue is the development of ulcers when cysteamine is used at high doses (generally >140 mg/kg) [24]. Cysteamine also results in developmental toxicities including embryo malformations, intrauterine growth retardation, and fetal death at doses that did not cause maternal toxicities [25]. Furthermore, it leads to skin, vascular, neurologic, and muscular problems, bone lesions [26], and copper deficiency [19]. Most of the patients with cystinosis on which cysteamine is used are young children and it is the only medicine available for treating this disease. Although cysteamine administration is known to be problematic, its effect on the reproductive system, especially the mammary glands, is currently unknown. The pubertal period is an important window for mammary gland development [27,28] and this is promoted by, estradiol (E) and progesterone (P). Moreover, growth factors produced in the mammary glands also play important roles in stimulating pubertal mammary gland development. These growth factors include insulin-like growth factor I (IGF-I) [29], amphiregulin (Areg, a member of the epidermal growth factor family and a ligand of epidermal growth factor receptor) [30], hepatocyte growth factor (HGF) [31–33], growth hormone (GH), and others. The objective of this investigation was to explore the effects of low dose of cysteamine on pubertal mammary gland development, and to examine the underlying mechanisms.

In this investigation it was found that cysteamine inhibited ovine mammary gland development at very low doses after 5 months of treatment; furthermore, it decreased plasma progesterone levels and HGF in the mammary glands, thus inhibiting ovine mammary gland development. Cysteamine should currently be prescribed with great caution and ways to overcome its limitations should be investigated.

2. Materials and methods

2.1. Animals

The experiment was conducted with pubertal female sheep at Shouguang Hongde Farmer Co., Weifang, China. Forty crossbred Small Han × Xi'mao female sheep (two months old) were equally divided into two groups: control and cysteamine treatments. The sheep were fed with a creep diet containing grass, crop straw, and vegetables, in addition to a basal diet (0.5 kg/sheep/day; 40% corn, 10% soybean meal, 25% palm meal, 10% corn starch residue, and 15% wheat bran). Sheep in the cysteamine treatment group received both the creep diet and basal diet supplemented with a commercial cysteamine feed additive (supplied by Kangdequan Co, Ltd, Hangzhou, China; containing 30% cysteamine hydrochloride with starch and dextrin as carriers for stabilization) at the equivalent of 10 mg pure cysteamine/kg body weight (BW)/day (≥15 mg pure cysteamine/kg BW is used for treating cystinosis) [1]. Sheep in the control group were fed the creep and basal diets with a blank carrier (starch and dextrin, equivalent to the weight fed to the cysteamine group). All animal experimental procedures followed the regulations of the animal ethics committee of Qingdao Agricultural University.

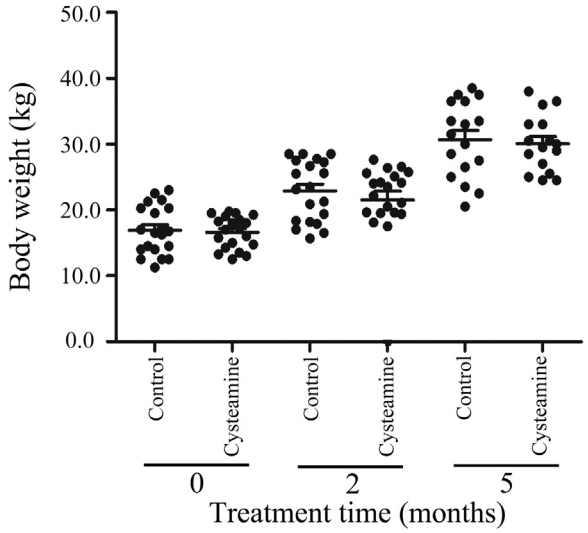

Fig. 1. Effects of cysteamine on sheep body weight. Cysteamine did not affect sheep body weight. The x-axis represents the treatment time (months), and the y-axis represents the body weight (kg).

2.2. Sample collection and H&E staining

Body weights were recorded every week. After 2 and 5 months of treatment, three sheep from the control and three from the cysteamine treatment were humanely killed. Tissue samples were collected and weighed post mortem. For each organ collected, part of the tissues were frozen at −80 °C, and part of the tissues were fixed in 10% neutral formalin and subsequently paraffin embedded. Subsequently, 5-μm sections were prepared and stained with hematoxylin and eosin (H&E). The mammary glands from each animal were collected, and cut into pieces in the same way. And two pieces closest to the nipple were fixed and embedded in the same way. Then the samples were cut to 5-μm sections. H&E sections of mammary glands were reviewed, blind to treatment, for treatment-related differences and pathological changes [27,28]. The duct structures [34] from five non-jacent sections were counted.

2.3. Routine blood test

Routine blood tests were performed to analyze the effects of cysteamine on blood cells using HEMAVET 950 (Drew Scientific Inc., FL, USA). Total blood samples were analyzed. The instrument was

Table 1
Organ index (% of body weight).

	Organ index (% of body weight)			
	Two months treatment		Five months treatment	
	Control	Cysteamine	Control	Cysteamine
Mammary gland	0.15 ± 0.02	0.12 ± 0.03#	0.16 ± 0.02	0.13 ± 0.01*
Ovary+uterus	0.04 ± 0.01	0.03 ± 0.01#	0.03 ± 0.005	0.03 ± 0.01
Kidney	0.13 ± 0.01	0.12 ± 0.01#	0.06 ± 0.004	0.06 ± 0.01
Liver	0.66 ± 0.06	0.77 ± 0.11#	0.70 ± 0.01	0.72 ± 0.01
Spleen	0.06 ± 0.01	0.07 ± 0.02	0.07 ± 0.001	0.08 ± 0.01
Heart	0.18 ± 0.03	0.20 ± 0.02	0.22 ± 0.02	0.22 ± 0.03

Note: #, $P < 0.1$; *, $P < 0.05$.