

The effect of mercury on thyroid function in sheep

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Key Words:

Sheep; mercury; thyroid function.

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Received: 23 February 2010,

Accepted: 11 October 2010

Abstract

The purpose of this experiment was to determine the effect of long-term low-dose administration of mercury (Hg) on thyroid function in sheep. In this experiment, 10 clinically healthy, adult, male Iranian sheep, aged approximately 1 year, were randomly allocated into the control (n=5) and mercury (Hg-)treated (n=5) groups. Both groups were kept under identical conditions in terms of food and environment. The treatment group received mercuric chloride (5 mg/kg/day) orally for eight weeks. Blood samples were drawn between the hours of 0800 and 0900 from both groups on days 0, 14, 28, 42, 56 and 70. Thyroid function was evaluated by measuring the levels of the serum thyroid hormones, including triiodothyronine (T3), thyroxine (T4), free T3 (FT3), free T4 (FT4) and thyroid-stimulating hormone (TSH). Hepatic function was evaluated in both groups by measuring alanine aminotransferase (ALT), aspartate aminotransferase (AST), -glutamyltransferase (GGT) and total bilirubin (TBIL). Renal function was assessed using serum creatinine (Cr) and blood urea nitrogen (BUN) levels. Serum T3 (from day 28 onwards) and serum T4, FT3, FT4 and TSH (from day 14 onwards) decreased in the Hg-treated group ($p < 0.05$). Serum ALT, AST and GGT increased from days 42, 56 and 70, respectively, when compared to the control group ($p < 0.05$). The concentrations of total protein and albumin decreased on day 70 and total bilirubin (TBIL), BUN and Cr levels increased on day 70 when compared to the control group ($p < 0.05$). It was concluded that chronic administration of Hg may expose sheep to the risk of hypothyroidism.

Introduction

Heavy metals have recently come to the forefront of the list of dangerous substances and are now considered to be serious chemical health hazards for humans and animals (Massadeh and Snook, 2002; Lars 2003). Mercury (Hg), a heavy metal of environmental concern, is widely considered one of the most toxic substances on earth (Clarkson, 1997) because elevated concentrations can cause toxicity in all living organisms (Gray and Hines, 2006). Hg has no known metabolic function and is not easily eliminated by humans or animals (Eisler, 1987). Animals may be exposed to Hg from contamination in air, soil or water, in addition to that which may be ingested with their feed (El-Hayek, 2007). Fossil-fuel combustion (Billings and Matson, 1972), smelting of commercial ores and use of agricultural fungicides may all contribute Hg to the environmental burden (NRC, 1980).

There is some evidence that Hg can affect the function of the thyroid gland (Nishida *et al.*, 1986;

Kawada *et al.*, 1980; Boas *et al.*, 2006; Tan *et al.*, 2009). The thyroid gland plays important roles in the regulation of energy usage, synthesis of RNA, consumption of oxygen by cells, overall body metabolism, growth processes and neurological development (Vanderpump and Tunbridge, 2008). Normal function of the thyroid gland and activity of thyroid hormones are considered crucial to sustain productive performance in domestic animals (growth, milk and hair production) and circulating thyroid hormones can be considered as indicators of the metabolic and nutritional status of the animals (Riis and Madsen, 1985; Todini *et al.*, 2007). In adult sheep, the most prominent clinical findings in hypothyroid cases are poor wool growth, depressed milk yield, reduced weight gain, impaired reproductive performance with loss of libido in rams and late abortions, the birth of weak lambs with visibly enlarged thyroid glands and an increased susceptibility to infectious agents (Sipos *et al.*, 2004). As there is a lack of literature on the effect of Hg on the thyroid function

in sheep, the present study was carried out to evaluate its effects on some of the basic thyroid function tests and on vital organs such as the liver and kidneys.

Materials and Methods

In this experiment, 10 clinically healthy, adult, male, mixed breed Iranian fat-tailed sheep, aged approximately 1 year, were randomly allocated into two equal groups: the controls (n=5) and the Hg-treated (n=5) groups. The two groups were kept under identical conditions in terms of both food and environment. Water and hay were supplied freely to all animals. Before commencing the experiment, samples of blood and feces were submitted for hematological, biochemical and parasitological examinations to assess the health status of the animals. The treatment group received 5 mg/kg/day mercuric chloride (HgCl₂) orally for eight weeks. Blood samples were drawn between the hours of 0800 and 0900 from both groups on days 0 (before receiving HgCl₂ in the treatment group), 14, 28, 42, 56 and 70. Samples were collected by jugular venepuncture into sterile, silicone-coated vacutainers, allowed to clot and then centrifuged at 3,000 rpm for 10 min. Serum was separated and stored at -20°C until required. Serum triiodothyronine (T3), thyroxine (T4), free T3 (FT3), free T4 (FT4) and thyroid-stimulating hormone (TSH) were measured by radioimmunoassay (RIA) (Kaneko, 1997). Serum samples were digested and Hg was measured by atomic absorption spectrophotometry (Shimadzu AA-670; Shimadzu Corp, Kyoto, Japan) at a wavelength of 228.8 nm (Szkoda and mudzki, 2005). All glassware was acid-washed with 2 M nitric acid. Standards were prepared from a certified Hg-standard solution. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured by a modified Reitman-Frankel method.

Blood urea nitrogen (BUN) was measured by the diacetyl monoxime method. Serum creatinine (Cr) was measured using Jaff's method (Myers *et al.*, 2006). Serum ALT, AST, BUN, Cr and -glutamyltransferase (GGT) were measured by COBAS MIRA autoanalyser (Roche Instrument, UK). Total bilirubin (TBIL) was measured using Roche Diagnostic Kits (Hoffman-La Roche Co. Ltd., Diagnostica, Basel, Switzerland).

Data were checked for normality before performing analyses. All analyses utilized parametric statistical methods. Results were expressed as mean ± SE. All data were analyzed using SPSS for Windows, Version 15.0. In order to compare the mean value for each parameter in the control group with that for the same parameter in the treatment group on each day, repeated measure ANOVA and Bonferroni post hoc tests were used. The level of statistical significance for each test was set at p<0.05.

Results

The results are shown in Tables 1 and 2. The serum Hg concentration increased in the treatment group from day 14 onwards (p<0.05). The serum T4, FT3, FT4 and TSH concentrations decreased in the treatment group from day 14 onwards and serum T3 decreased from day 28 (p<0.05). The serum levels of ALT (from day 42), AST (from day 56) and GGT (day 70) increased when compared to the control group (p<0.05). The concentrations of total protein and albumin decreased on day 70, and TBIL, BUN and Cr levels increased significantly on day 70 compared to control values.

Discussion

The thyroid gland produces hormones that are essential for the regulation of normal body metabolism, and serum levels of these hormones are considered as

Table 1: Concentrations (mean ± SE) of thyroid hormones before and following Mercuric Chloride administration in sheep (n=10).

Parameters		Sampling days					
		0	14	28	42	56	70
T3 (nmol/L)	Treatment	1.14±0.10 ^a	1.28±0.12 ^a	0.63±0.14 ^{b*}	0.53±0.10 ^{b*}	0.52±0.12 ^{b*}	0.47±0.11 ^{b*}
	Control	1.13±0.11	1.33±0.17	1.06±0.11	1.06±0.18	1.08±0.08	1.13±0.11
T4 (nmol/L)	Treatment	83.26±4.90 ^a	31.00±4.72 ^{b*}	27.00±4.12 ^{b*}	33.00±3.77 ^{b*}	28.00±4.71 ^{b*}	30.00±4.57 ^{b*}
	Control	83±4.81	88±5.05	87±7.03	83±5.00	76±5.05 ^f	83±4.61
FT3 (pmol/L)	Treatment	5.94±0.50 ^a	2.03±0.35 ^{b*}	1.90±0.37 ^{b*}	1.70±0.42 ^{b*}	1.50±0.27 ^{b*}	2.40±0.32 ^{b*}
	Control	5.98±0.43	6.75±0.51	5.90±0.45	6.10±0.35	5.08±0.60	5.97±0.43
FT4 (pmol/L)	Treatment	30.00±1.15 ^a	17.40±1.64 ^{b*}	19.00±1.1 ^{b*}	22.40±1.26 ^{b*}	12.20±1.37 ^{b*}	15.80±1.26 ^{b*}
	Control	30±1.03	29±1.45	32±2.70	31±2.45	27±2.58	30±1.02
TSH (mIU/L)	Treatment	0.12±0.01 ^a	0.01±0.00 ^{b*}	0.02±0.01 ^{b*}	0.01±0.02 ^{b*}	0.02±0.01 ^{b*}	0.03±0.01 ^{b*}
	Control	0.13±0.01	0.11±0.02	0.14±0.02	0.14±0.01	0.14±0.02	0.13±0.01

*Significantly different (p<0.05) vs. control. In each row, in treatment group, different letters (a and b) indicate significant differences among the mean of specified parameter in different days. (T3, Triiodothyronine; T4, Thyroxine; FT3, free T3; FT4, free T4; TSH, Thyroid-Stimulating Hormone)

Table 2: Concentrations (mean \pm SE) of serum Mercury (Hg), Total Protein (TP), Albumin (Alb), Total Bilirubin (TBIL), Blood Urea Nitrogen (BUN) and Creatinine (Cr) and Serum Hepatic Enzyme levels before and following Mercuric Chloride administration in sheep (n=10).

Parameters	Sampling days						
	0	14	28	42	56	70	
Hg (μ mol/L)	Treatment	0.00 \pm 0.00 ^a	0.55 \pm 0.03 ^{b*}	0.20 \pm 0.02 ^{c*}	0.10 \pm 0.01 ^{c*}	0.14 \pm 0.01 ^{c*}	0.13 \pm 0.02 ^{c*}
	Control	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
ALT (IU/L)	Treatment	36.60 \pm 1.69 ^a	27.66 \pm 2.73 ^a	31.00 \pm 2.10 ^a	105.00 \pm 3.60 ^{b*}	80.00 \pm 3.40 ^{b*}	105.00 \pm 3.25 ^{b*}
	Control	38 \pm 2.35	41 \pm 3.25	39 \pm 1.94	34 \pm 2.40	39 \pm 4.50	38 \pm 2.35
AST (IU/L)	Treatment	105.0 \pm 2.10 ^a	105.0 \pm 4.23 ^a	101.0 \pm 3.40 ^a	108.1 \pm 5.6 ^a	184 \pm 9.12 ^{b*}	133.0 \pm 3.70 ^{c*}
	Control	103 \pm 3.06	93 \pm 3.60	109 \pm 8.51	116 \pm 6.35	95 \pm 4.6	103 \pm 3.43
GGT (IU/L)	Treatment	52.6 \pm 6.31 ^a	40.3 \pm 2.96 ^a	61.0 \pm 3.12 ^a	53.0 \pm 3.52 ^a	49.0 \pm 4.70 ^a	115.0 \pm 5.00 ^{b*}
	Control	61.2 \pm 3.33	52.0 \pm 3.50	78.2 \pm 4.62	59.4 \pm 5.45	57.0 \pm 5.50	61.4 \pm 3.40
TP (g/L)	Treatment	66.2 \pm 8.48 ^a	63.0 \pm 7.43 ^a	61.7 \pm 7.53 ^a	59.6 \pm 7.55 ^a	58.9 \pm 6.40 ^a	53.4 \pm 6.40 ^{a*}
	Control	64.2 \pm 7.23	62.5 \pm 6.50	63.7 \pm 8.72	65.6 \pm 6.47	62.9 \pm 7.51	61.4 \pm 8.3
Alb (g/L)	Treatment	24.7 \pm 3.44 ^a	25.56 \pm 3.50 ^a	25.8 \pm 4.22 ^a	25.1 \pm 4.81 ^a	22.5 \pm 5.3 ^a	18.1 \pm 4.3 ^{b*}
	Control	25.4 \pm 2.61	24.9 \pm 3.1	26.5 \pm 3.6	26.8 \pm 3.35	24.3 \pm 3.40	25.9 \pm 5.60
TBIL (μ mol/L)	Treatment	6.32 \pm 0.34 ^a	4.78 \pm 0.34 ^a	5.13 \pm 0.51 ^a	9.97 \pm 0.54 ^b	10.08 \pm 0.7 ^b	12.19 \pm 0.4 ^{b*}
	Control	6.15 \pm 0.17	6.15 \pm 0.61	5.81 \pm 0.51	7.84 \pm 0.34	8.15 \pm 0.51	7.32 \pm 0.34
BUN (mmol/L)	Treatment	7.11 \pm 0.68 ^a	7.48 \pm 0.59 ^a	7.73 \pm 0.8 ^a	8.2 \pm 0.71 ^a	7.9 \pm 0.86 ^a	11.65 \pm 0.98 ^{b*}
	Control	6.4 \pm 0.74	7.3 \pm 0.65	7.46 \pm 0.78	7.1 \pm 0.63	6.8 \pm 0.59	7.52 \pm 0.7
Cr (μ mol/L)	Treatment	78.2 \pm 0.01 ^a	80.3 \pm 9.4 ^a	83.4 \pm 10.1 ^a	85.9 \pm 10.5 ^a	81.1 \pm 9.03 ^a	95.4 \pm 12.37 ^{b*}
	Control	82.1 \pm 9.32	79.6 \pm 10.2	85.1 \pm 9.95	81.4 \pm 11.6	77.6 \pm 8.4	83.9 \pm 10.2

*Significantly different ($p < 0.05$) vs. control. In each row, in treatment group, different letters (a and b) indicate significant differences among the mean of specified parameter in different days. (ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, -glutamyltransferase)

reliable indicators of thyroid function in both humans and animals (Paier *et al.*, 1993; Chaurasia *et al.*, 1996; Kelly, 2000). All of the reactions necessary for the formation of T3 and T4 in the follicular cells of the thyroid gland are controlled by TSH released from the pituitary (Kelly, 2000). TSH secretion is modulated by the circulating levels of FT4 and FT3 and by the conversion of T4 to T3 in the pituitary thyrotropic cells. T3 is the metabolically active hormone. Thyrotropin-releasing hormone (TRH), a 3-amino acid peptide synthesized in the hypothalamus, also stimulates the pituitary to release TSH (Kelly, 2000; Higgins, 2007). In our study, mean serum concentrations of T3, T4, FT3, FT4 and TSH decreased significantly in Hg-treated sheep. Nishida *et al.* (1986) suggested that thyroidal secretion of T4 was inhibited by Hg. Kawada *et al.* (1980) indicated that both organic and inorganic Hg disrupted with thyroidal function by interfering both with the production of thyroidal hormones and the conversion of T4 to T3. Björkman *et al.* (2007) reported significant correlations between the number of amalgam-filled tooth surfaces and Hg concentrations in the pituitary and thyroid glands. High concentrations of inorganic Hg in the pituitary were also reported after experimental exposure of monkeys to methyl mercury (Vahter *et al.*, 1995).

One possible explanation for the thyroid dysfunction found in our study might be the accumulation of Hg in the thyroid gland. Since the thyroid gland is the only organ involved in T4 synthesis, the decrease in the serum level of this hormone in the Hg-treated sheep could suggest that Hg-induced thyroid dysfunction is due to alterations in the production and/or secretion of T4 by the follicular cells of thyroid gland. Although the levels of free thyroid hormones were reduced in our Hg-exposed sheep, the normal TSH response to low thyroid hormones did not occur and conversely a significant decrease in hormone levels was observed. This significant decrease in TSH levels in the Hg-treated sheep could also be the result of the effect of Hg on the regulatory enzymes associated with the hypothalamic-pituitary-thyroid (HPT) axis. Alternately, since thyroid hormones are metabolized in the peripheral tissues by deiodination, conjugation, deamination and decarboxylation enzymatic reactions, alterations in these metabolic pathways may significantly affect thyroid hormone metabolites and influence thyroid function at the cellular level (Paier *et al.*, 1993; Chaurasia *et al.*, 1996; Kelly, 2000).

Most of the circulating T3 originates from extra-thyroidal tissues. The peripheral deiodination of T4 to T3, which takes place mainly in the liver, is dependent

on the activity of 5-monodeiodinase (5-D) (Paier *et al.*, 1993; Chaurasia *et al.*, 1996; Piłat-Marcinkiewicz *et al.*, 2003). A common feature of thyroid hormone dysfunction at the cellular level is a low level of circulating T3, generally with a normal to slightly elevated serum T4 level, and either normal or slightly suppressed TSH level (Kelly, 2000).

The significant increase in the serum levels of ALT, AST, GGT, TBIL, BUN and Cr in the Hg-treated sheep in our study could be an indication of injury to hepatic cells (Latimer *et al.*, 2003) and the kidneys. Hepatic pathology may influence concentrations of the serum thyroid hormones because of its effect on the enzymes of the metabolic pathways (Kelly, 2000). Therefore, the decrease of serum T3 concentration in the Hg-treated sheep might be related in part to the observed hepatic dysfunction.

The kidneys are the major target organs for inorganic Hg in humans and in experimental animals (Klaassen, 2001; Liu *et al.*, 2007). Sharif *et al.* (2005) showed that the highest concentration of Hg in sheep tissues was found in the kidneys, followed by liver and the muscles. Other authors also confirmed that Hg concentrated mainly in the kidneys (Falandysz, 1991; Reykdal and Thorlacius, 2001; Cang *et al.*, 2004). Toxic nephropathy has been described in sheep (Robinson and Hesketh, 1976) and goats (Kumar and Pandey, 1994; Pathak and Bhowmik, 1998) with induced chronic Hg poisoning. The kidneys normally play an important role in the metabolism, degradation and excretion of several thyroid hormones. Chronic renal failure (CRF) can affect thyroid function in many ways, including low circulating thyroid hormone levels, altered peripheral hormone metabolism, insufficient binding to carrier proteins, possible reduction in tissue hormone content and altered iodide storage in the thyroid gland (Lim, 2001). The kidneys normally contribute to the clearance of iodide, primarily by glomerular filtration. Therefore, iodide excretion is diminished in advanced renal failure, which leads to an elevated concentration of plasma inorganic iodide and an initial increment in iodide uptake by the thyroid gland. The ensuing marked increase in the intrathyroidal iodide pool results in diminished uptake of radiolabeled iodide by the thyroid in uremic patients (Ramirez *et al.*, 1973). Increases in total body inorganic iodide can potentially block thyroid hormone production, which is known as the Wolff-Chaikoff effect (Kaptein, 1996).

In conclusion, it seems likely that the hypothyroid state in sheep that are chronically exposed to Hg is multifactorial, with contributing factors that include Hg accumulation in the thyroid gland, and hepatic and renal involvement.

Acknowledgment

This work was sponsored by a grant from Shiraz University, Iran, No. 84-VE-1784-C308.

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