Histological evaluation of the thyroid structure after co-exposure to cadmium and ethanol

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Abstract

The aim of this study was to evaluate the influence of co-exposure to cadmium (Cd) and ethanol on the structure and function of the thyroid. Male Wistar rats were exposed to 50 mg of Cd/dm^3 in drinking water and ethanol in a dose of 5 g/kg body wt/24 h (administered intragastrically in two equal doses for 5 days a week) for 12 weeks. The structure of the thyroid was assessed in a light microscope. Immunohistochemical methods were used to determine calcitonin (CT), the calcitonin-gene related peptide (CGRP), somatostatin (ST) and synaptophysin (SPh) in the thyroid parafollicular cells (C cells). Weakening of the reactions for CT, CGRP, ST, SPh was observed in C cells. The animals, exposed to a combined action of Cd and ethanol, showed signs of enhanced activity (elevated light follicular epithelium and rarefied colloid), as well as features of intensified remodelling (partial or total follicular atrophy and the appearance of new follicles) of the thyroid gland. In some fragments of the connective tissue stroma mononuclear cell infiltration was observed. The nature of the changes, observed in the rats, simultaneously exposed to Cd and ethanol, may suggest an enhancement in the function of C cells.

Key words: cadmium, ethanol, thyroid, morphology, immunohistochemistry, rat.

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Introduction

Cadmium (Cd), one of the most toxic heavy metals, is a major chemical environmental pollutant. Cigarette smoking can be an essential source of exposure to this metal. Prolonged exposure to Cd poses a serious threat to humans and animals [1, 2], as Cd can damage various organs and tissues, especially the kidneys, the lungs, the testes and bones [1, 2]. Literature data and our own findings have provided some evidence that Cd can also affect the thyroid which an important regulatory gland [3, 4]. Also alcoholism is a serious problem in almost all countries [5]; however, not many data are available, regarding the effect of ethanol on the thyroid. Ethanol abusers can simultaneously be exposed to substantial amounts of Cd, due to its presence in the human environment, in tobacco smoke and, frequently, at work [1, 2]. No evidence has been found in the available literature on the structure and function of the thyroid at co-exposure to both substances. Since Cd and ethanol can damage the structure and function of the thyroid, and the functional state of this gland is known to affect ethanol metabolism, changes that can be induced by these substances, administered jointly, are difficult to predict. Therefore, we decided to examine the structure and function of the thyroid at co-exposure to Cd and ethanol.

Material and methods

Fourteen 8-weeks old male Wistar rats of 170g initial body weight were used in the study. The animals were housed under controlled conditions (temperature: 22 ± 2^{0}C, relative humidity: 50 ± 10%, natural light-dark cycle) and had a free access to standard rodent laboratory LSM chow (Argopol, Motycz, Poland) and drinking water. The animals were randomly divided into two groups of seven rats in each. One group was exposed to 50 mg Cd/dm^3 (as cadmium chloride) in drinking water and ethanol in a dose of 5 g/kg body wt/24 h (administered intragastrically in two equal doses for 5 days a week) for 12 weeks.

Table 1. Changes, observed in the thyroid structure of animals exposed to cadmium and ethanol

Change	Intensity degree of changes and the frequency of their occurrence		
	+	++	+++
Remodelling of glandular structure of the thyroid	0	5*	2
Mononuclear cell infiltration in connective tissue	0	7	0
Rarefied colloid	0	5	2
Elevated epithelium, light cytoplasm of follicular cells	0	5	2

The following changes were observed: + - only in some follicles of the thyroid, ++ - in some fragments of the thyroid, +++ - almost in all the fragments of the thyroid, *the number of animals in which a change was observed.

Table 2. Intensity of immunohistochemical reactions in C cells of the thyroid

Group	n	ST	SPh	CT	CGRP
Control	7	+++	+++	+++	+++
Cd+ethanol	7	+	+++ or ++*	++	++

Reactions were performed in 7 animals of each group. +++ -strong reaction in most of C cells, ++ -weakening of the reaction in most C cells, + - general weakening of the reaction in C cells, * ++ - weakened reaction was observed in some animals only.in all the fragments of the thyroid, *the number of animals in which a change was observed.

Figure 1. Anti-CGRP antibodies induce strongly immunopositive reaction in most C cells of control rats. (mag. x150).

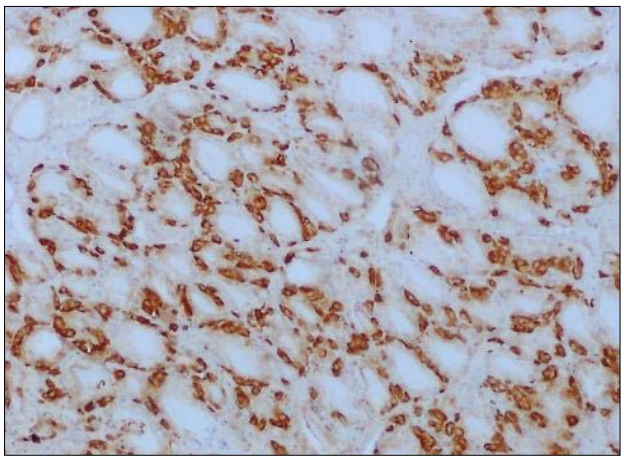


Figure 2. Most of C cells of the control thyroids show strongly immunopositive reactions for CT (mag. x300).

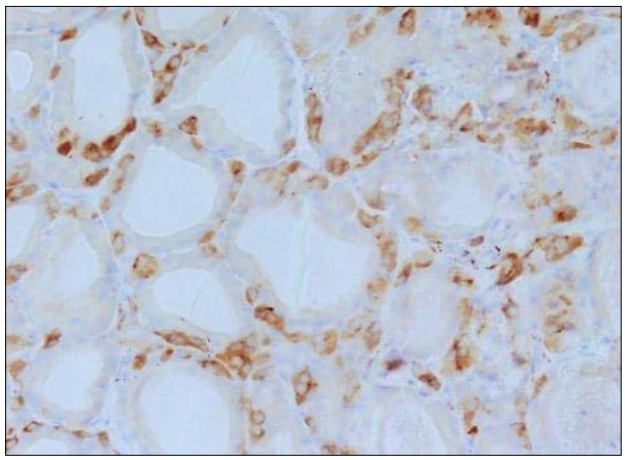


Figure 3. Weak reaction for CGRP in C cells of the rats exposed to Cd + ethanol (mag. x300).

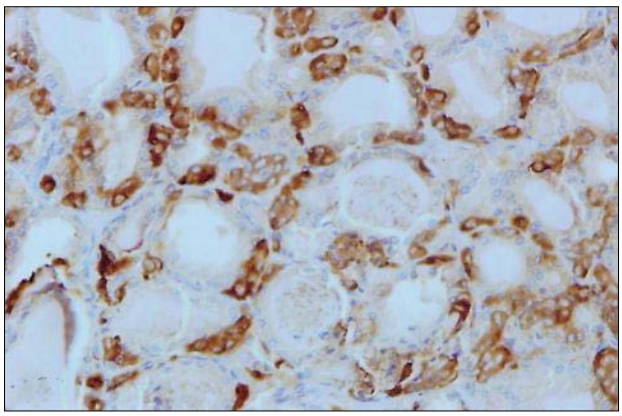
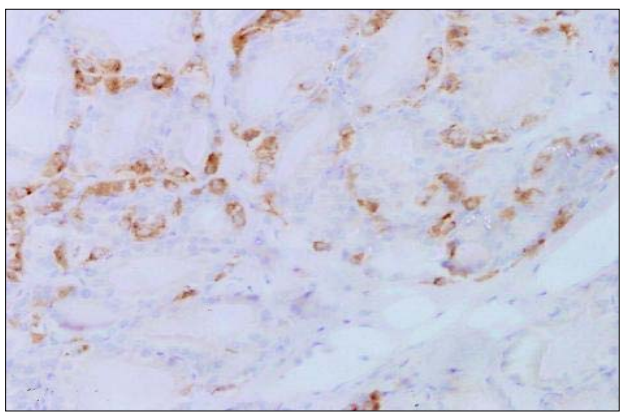


Figure 4. Most of C cells of the Cd + ethanol-exposed rats show a weak reaction for CT (mag. x300).



The second group, which drank water free of Cd, served as control. At the end of that study, both thyroid lobes (together with parathyroids) were collected under anaesthesia and immediately fixed in Bouin's fluid for 24 hours. Next, the thyroid lobes were embedded in paraffin, cut into 5-6 mm sections, routinely stained with hematoxylin and eosin (H+E) and examined in a light microscope (NIKON ECLIPSE E 400, USA). Moreover, silver impregnation was performed for the identification of C cells [6]. The assessment of changes in thyroid structure included a determination of their intensity, according to the following criteria: + - a change occurs in some thyroid follicles only, ++ - in some thyroid fragments or +++ - in almost all thyroid fragments. Moreover, immunohistochemical reactions (the avidin-biotin technique) [7] for the hormones produced by C cells, such as calcitonin (CT), the calcitonin-gene related peptide (CGRP) and somatostatin (ST), as well as functionally important protein - synaptophysin (SPh) were performed on thyroid paraffin sections, using specific rabbit antibodies (DAKO).

The study was approved by the Local Ethics Committee for Animal Experiments in Bialystok (Poland).

Results and discussion

Microscopic analysis of the thyroid revealed some histological changes in the animals subjected to co-exposure to Cd and ethanol. Some fragments showed remodelling of the glandular structure of the thyroid, an inflow of mononuclear cells and alterations in the structure of the epithelial lining of follicles. Only in two rats, those changes occurred in almost all the fragments of the thyroid (Table 1). In the control animals, as revealed by immunohistochemical examinations, the antibodies, used against CGRP (Fig. 1), CT (Fig .2), SPh and ST, reacted with respective antigens, inducing a strongly positive reaction in the majority of C cells. In the Cd + ethanol exposed animals, all the reactions were weakened (Table 2), especially those for CGRP (Fig. 3) and CT (Fig. 4), in comparison to respective values in the control. The immunohistochemical reaction for ST was found in a considerably smaller number of cells, compared to that in the control. The reaction for SPh was weakened in some animals only. The intensity of reactions with silver salts in C cells was similar in control and experimental animals. The above data demonstrate effects of Cd and ethanol on the structure and the functional state of the thyroid. Previously, we found that Cd induced changes within the structure of follicular cells and follicles, what can suggest thyroid function disorders already at the level, corresponding to the human environmental exposure (5 mg Cd/dm^3). Most of the data, which are available on toxic effects of ethanol on the thyroid structure, have been obtained from experiments, in which ethanol was injected directly to this gland [8, 9]. We have found some histological changes and disturbances in the secretory function of the thyroid in rats after oral ethanol administration [10]. The changes, observed in the animals exposed to Cd + ethanol in the present study, resemble those described previously after the exposures to Cd or ethanol alone, although, in intensity, they are similar to those induced by Cd alone. The nature of histological changes in the animals exposed to a combined effect of Cd and ethanol suggests an enhanced activity of the thyroid gland. The weakened immunohistochemical reaction for CT, CGRP and ST might result from their increased secretion. The issue needs further studies.

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