Acta Veterinaria (Beograd), Vol. 61, No. 5-6, 479-488, 2011.

DOI: 10.2298/AVB1106479M UDK 612.1/.8.112/44:599.1.3./.8.4

PHENOTYPE OF BLOOD LYMPHOCYTES IN CORRELATION WITH HISTOLOGICAL PICTURE IN THYROID GLAND OF RATS TREATED WITH POTASSIUM IODIDE

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(Received 4th April 2011)

Having in mind the former results which confirmed the functional relationship between the thyroid and the immune system, and the administration of potassium iodide (KI) in the therapy of auto-immune diseases, we considered it of interest to investigate the pathogenesis of KI induced experimental thyroiditis, by studying the morphology of thymus, thyroid gland and spleen, as well as the phenotype of lymphocytes in the thymus and peripheral blood in relation to the expression of CD4 and CD8 molecules. The experiments have been carried out on 30 male Wistar rats, divided in 3 groups. The first group (n=10) received KI (225 μg/g, i.p.), the second group (n=10) received KI (675 μg/g, i.p.), while the third group (n=10) received sodium chloride (0.9%, i.p.). The intensity of histological lesions in the thyroid gland, was statistically significant ($p<0.01$) in KI treated groups, compared to the controls. KI also significantly decreased rat body mass, and increased masses of thymus and thyroid gland ($p<0.05$). No statistically significant difference was found in thymocyte and peripheral blood CD4+ and CD8+ subpopulation numbers between the groups. Our experiments suggest that KI, at least in some doses could induce mild lymphocytic thyroiditis in rats, and that this simple, practical and non-expensive model of experimental thyroiditis could be of importance for further research.

Key words: CD4, CD8, potassium iodide, thymus, thyroiditis

INTRODUCTION

Many researchers state that the autoimmune thyroid diseases (AITD), Graves' disease and chronic lymphocytic thyroiditis (CLT) are today amongst the most common endocrine diseases in childhood and adolescence (Brown, 2009). Many physicians agree that genetic factors play a major role in their etiology, and in the past, a couple of susceptibility genes have been identified (Dultz *et al*.,

2009). It has been shown that thyroid cell destruction in autoimmune hypothyroidism is dependent on T cell-mediated cytotoxicity with the likely additional effect of receptor-mediated apoptosis (Stojanović *et al*., 2009). Also, some findings suggest that the thyroid cell destruction characteristic of autoimmune thyroiditis can be seen as the consequence of inappropriate expression of Fas or TRAIL death pathway molecules and down-regulation of the apoptosis controlling protein Bcl-2, which may be induced by cytokines, locally released by infiltrating lymphocytes (Stojanović *et al*., 2009). Most autoimmune thyroid diseases, such as Graves' disease and Hashimoto's thyroiditis, are characterized by profuse infiltrates of both CD4+ and CD8+ T cells (Martin *et al*., 1999). Altered expression of T lymphocyte surface markers is also associated with chronic autoimmune thyroiditis, and lymphocyte activation in those patients leads to some changes in the proportion of T cell subsets (Kucharska *et al*., 2008).

Potassium iodide (KI) is a compound made of 76% of the halogen iodine and 23% of the alkali metal potassium by weight (Sterling and Heymann, 2000). Today, it is frequently used for inflammatory dermatoses treatment. The precise mechanism by which KI acts is unknown (Sterling and Heymann, 2000).

Several studies have suggested that iodine may influence thyroid hormone status, and perhaps antibody production, in patients with autoimmune thyroid disease (Reinhardt *et al*., 1998). Also some authors have found that in autoimmune-prone mice, excessive iodine administration might induce goiter, thyroiditis, worsen lymphocytic infiltration, as well as damage the thyroid follicular structure in a dose-dependent manner (Teng *et al.*, 2009). Also, experimental thyroiditis induced by administration of supraphysiologic doses of iodine has been described in nonobese diabetic (NOD) mice or their derived strains such as NOD.H.2h4 (Boechat *et al.*, 2002; Damotte *et al*., 1997), and in other models of thyroid autoimmunity, such as BB/W rats (Li and Boyages, 1994), and obese strain chickens (Sundick *et al*., 1996).

Having in mind the former results which confirmed the functional relationship between the thyroid and the immune system, and the administration of KI in the therapy of some auto-immune diseases, we considered it of interest to investigate the pathogenesis of KI induced experimental thyroiditis, by studying the morphology of the thymus, thyroid gland and spleen, as well as the phenotype of thymocytes and peripheral blood T-cells in relation to the expression of CD4 and CD8 molecules.

MATERIAL AND METHODS

Animals

The study was carried out on 30 male Wistar rats (m=99-120 g), aged six to eight weeks, obtained from the Experimental Animals Farm of the Military Medical Academy and from the Pathologic Physiology Institute of the Faculty of Medicine University of Belgrade. All animals have been weighed on two-day intervals during experiments. Within the animal facility, environmental conditions such as temperature, humidity, light and noise were regulated in order to establish consistency. The investigation conforms to the Guide for the Care and Use of

Laboratory Animals published by the U.S. National Institute of Health (NIH Publication No. 85 – 23, revised 1985).

Experimental groups

The animals were divided in 3 groups. The first group (n=10) received KI (225 μg/g, i.p.), the second group (n=10) received KI (675 μg/g, i.p.), while the third control group (n=10) received sodium chloride (0.9%, i.p.).

Quantitative analysis of histological lesions

The thymus, the thyroid and the spleen in all animals were removed, measured (volume and mass) and fixed in Carnoy solution or in 10% neutral formalin. After fixation, the 2 μm thick paraffin tissue sections were stained by haematoxylin and eosin and/or by Methyl-green pyronine and were analyzed using light microscopy (Olimpus-2 microscope). The intensity of histological lesions had been graded from 0 to 4 as previously described (Sugihara *et al.*, 1990) in the following manner: 0 – normal structure of the gland; 1 – one or two focuses of infiltration; 2 – three or more focuses of infiltration; 3 – diffuse infiltration with scattered destruction of the follicles; 4 – diffuse infiltration with massive destruction of the follicles and infiltration by connective tissue.

Blood samples

Blood was obtained for immunological analysis and the animals were sacrificed by cardiac puncture on the 26th day after the beginning of the experiment.

Flow cytometer (FACScan) analysis of lymphocyte subsets

In order to determine lymphocyte subsets we used double immunofluorescence labeling of CD4 and/or CD8 molecules. T-cell subpopulations were determined using monoclonal antibodies: phycoerythrin (PE) marked with anti-W3/25 (CD4), and fluoresceinisocyanate (FITC) marked anti-MRC OX8 (CD8) - Serotec (Oxford, England). Double immunofluorescence cell staining and analysis were performed according to Itojama (1989) and Shivakumara (1989). Briefly, 2x 10^5 to 10^6 cells were suspended in 100 μL of PBS containing 2% FCS and 0.01% sodium azide, were sequentionally incubated for 30 min on ice with FITC- conjugated anti -CD8 and PE-conjugated anti –CD4 mAbs. Specificity of staining, and efficiency of blocking were controlled using isotype-matched mAb. To perform triple color analyses, cells were incubated sequentionally for 30 min with FITC- conjugated anti -CD8 and PE-conjugated anti –CD4, and biotin-conjugated anti – TCR (α/β) mAbs, using streptavidin conjugated duochrome. In experiments, 2x10^4 flow cytometer events were analyzed using *Consort 30* and *Facscan* research software. All data were collected and displayed on a log scale of increasing green and red fluorescence intensity. The cell subpopulations had been analyzed on FACScan (Becton Dickinson) in the program *Consort 30.*

Statistical analysis

Results are shown as the mean (X) and standard deviation (SD). Statistical analysis of the results was performed using one-way analysis of variance (ANOVA, Statistica, version 7.1.). The differences with values of $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

In the past, there have been many studies, which used various animal models of experimental thyroiditis to examine autoimmune disorders (Chen *et al*., 2009; Choi *et al*., 2008; Sundick *et al*., 1996; Tan *et al*., 2008). Some of these studies used bovine or human thyroglobulin immunization (Arata *et al*., 2006; Choi *et al.*, 2008), or immunization with recombinant thyroid peroxidase (Ng *et al*., 2006). Others used mouse thyroglobulin in Freund's complete adjuvant (FCA) or lipopolysaccharide (Cihákova *et al*., 2004). Another model of experimental thyroiditis that has recently been used mice that express certain alleles on an NOD genetic background which makes them predisposed to develop spontaneous thyroiditis, which is exacerbated with dietary iodine (Cihákova *et al*., 2004).

KI significantly decreased the body weight of the rats compared to the control group ($p<0.05$, Table 1). The significant body mass decrease in the groups treated with KI might have been due to possible rise in thyroid gland activity, hyperthyroidism, as in subacute lymphocytic thyroiditis with transient hypertyroidism, or in 5-10% of patients on the beginining of chronic lymphocytic (Hashimoto's) thyroiditis in humans (Dillmann, 2000) and subsequent increase in basal metabolism. However, we should have in mind that body mass depends on many variables, and basal metabolism is only one of them.

Table 1. Masses of rats (g) treated with KI and of the controls

n	KI 225 μg/g	KI 675 μg/g	NaCl
1	262	242	280
2	250	242	310
3	270	228	347
4	290	250	334
5	260	254	380
6	240	232	330
7	270	260	295
8	300	218	320
9	302	234	300
10	278	258	232
x	272.2	241.80	312.8
SD	20.56	13.84	40.24

The histological analyses of thymus, spleen and thyroid have shown significant region specific changes in the thyroid. KI significantly increased thyroid gland mass ($p<0.05$), similar as thyroid enlargement wich is the most frequent manifestation in Hashimoto's thyroiditis. On the section of the thyroid of rats that received KI, diffuse mononuclear cell infiltration with lymphocytes and just a few plasma cells in the follicles and in the spaces between the follicles with the destruction of gland acini and connective tissue proliferation have been seen. The intensity of histological lesions were statistically significant ($p<0.01$, Table 2, Fig. 1) compared to the control group. The pathology of the thyroid gland is characterised by extensive fibrosis throughout the gland, very similar to fibrosis in chronic lymfocitic thyroiditis (Brown, 2009). Our experiments have shown that KI, at least in some doses, induces mild lymphocytic thyroiditis in rats with body weight loss.

Table 2.Glandular mass index (mg/g)

KI 225 μg/g	KI 675 μg/g	NaCl
0.063	0.085	0.053

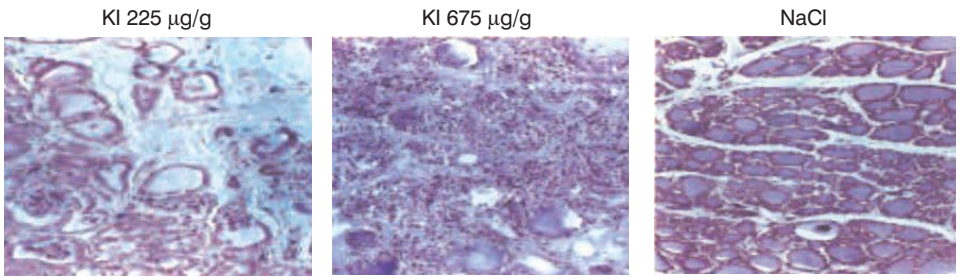

Figure 1. Histological lesions in thyroid gland in KI 225 μg/g, KI 675 μg/g treated rats and the controls after 26 consecutive days (HE, 40X)

The exact pathophysiological mechanisms that are responsible for this KI action are not fully understood. Some authors suggest that KI may trigger thyroid autoimmune reactivity by increasing immunogenicity of Tg, or by inducing damage to the thyroid and its cells by free radicals (Braverman and Utiger, 2005). We can agree with those authors having in mind one of the mechanisms for autoimmunity - sequestered antigen Tg after damaging glandular follicles became 'visible' for immunocompetent cells. So, because the immune system does not have immunologic tolerance to sequestered antigens, such as sperm, lens and Tg, autoimmune proceses started by lymphocyte infiltration which destroyed the normal follicular architecture. The question is what is the real pathway of the first event, i.e. the cell membrane destruction? The epitelial cells of the thyroid activly transport iodide. Iodine by itself, is the first known blocker of thyroid function, and can make reactive radicals after administration in high doses. Only iodide is permanently retained in the thyroid cell by organification.

Also 23% of the alkali metal potassium have tissue destructive potential in combination with hydroxy ions (Table 3).

Thymus mass was significantly higher ($p<0.05$) in rats treated with KI compared to the controls (Table 4). KI did not influence the thymus and spleen histological appearance in rats. Enlargement of the glandular mass of thyroid gland, perhaps, is because of fibrosis and cell infitration, but increase of thymus mass occurs because of higher water amount based on osmotic effect of K and I ions.

Table 3. Intensity of histological lesions in the thyroid gland in KI treated rats and controls

n	KI 225 μg/g	KI 675 μg/g	NaCl
1	4	2	0
2	3	1	0
3	3	2	1
4	1	2	1
5	1	3	1
6	4	2	0
7	2	3	0
8	3	3	0
9	3	4	1
10	2	3	1
X	2.60	2.50	0.50

Table 4. Thymus masses (mg) in KI treated rats and controls

n	KI 225 μg/g	KI 625 μg/g	NaCl
1	0.849	0.575	0.580
2	0.820	0.647	0.590
3	0.876	0.506	0.410
4	0.967	0.622	0.600
5	0.634	0.808	0.500
6	0.775	0.520	0.460
7	0.741	0.472	0.590
8	0.682	0.501	0.700
9	0.745	0.585	0.530
10	0.544	0.615	0.410
X	0.763	0.585	0.537
SD	0.123	0.098	0.093

Individual values of the surface phenotypes of lymphocytes stained with anti- CD4 and anti -CD8 monoclonal antibodies is shown in Table 5, Table 6 and Table 7. Administration of KI in the treatment of some auto-immune diseases (Sterling and Heymann, 2000), may find the explanation in a marked peripheral blood decrease in CD4+ numbers in KI treated groups (Table 5), but it was not statistically significant ($p>0.05$). No statistical significant difference has been detected in numbers of blood CD8+ T cells between the groups.

Table 5. Peripheral blood CD4+ in control and KI treated rats

n	KI 225 μg/g	KI 675 μg/g	NaCl
1	61.10	57.20	65.00
2	54.70	52.50	60.93
3	67.00	59.80	57.00
4	60.93	56.50	57.55
5	60.00	57.55	56.50
6	54.50	56.70	53.13
7	55.60	49.70	
8	60.10	53.00	
9	60.72	53.13	
X	59.40	55.12	58.35
SD	3.97	3.18	4.09

Table 6. Peripheral blood CD8+ subpopulation in control and KI treated rats

n	KI 225 μg/g	KI 675 μg/g	NaCl
1	21.70	35.50	35.00
2	17.50	34.50	27.73
3	44.00	26.30	30.23
4	27.73	32.10	26.35
5	23.90	26.35	32.10
6	30.20	23.60	25.73
7	30.70	26.40	
8	20.60	27.20	
9	26.35	25.73	
X	26.96	28.63	29.52
SD	7.77	4.26	3.60

Table 7. Peripheral blood CD4+/CD8+ subpopulation in control and KI treated rats

n	KI 225 μg/g	KI 675 μg/g	NaCl
1	2.82	1.61	1.86
2	3.13	1.52	2.20
3	1.52	2.27	1.89
4	2.49	1.80	2.18
5	2.51	2.26	1.76
6	1.80	2.40	2.06
7	1.81	1.88	
8	2.92	1.95	
9	2.26	2.06	
X	2.33	1.96	1.99
SD	0.55	0.30	0.18

Although we did not find a statistically significant difference in peripheral blood CD4+ and CD8+ subpopulation numbers between the groups, we cannot exclude the possibility that KI does have at least some morphological/immunological effects in the thymus and/or spleen, especially when we consider the reports from other authors (Teng *et al.*, 2009) which reported altered expression of T lymphocyte surface markers, lymphocyte activation and changes in proportion of T cell subsets associated with chronic autoimmune thyroiditis in the patients. The lack of statistical significance in our experiment could be due to the natural variability in rats. However, additional research might be needed to confirm this assumption.

Our experiments suggest that KI, in some doses induces mild lymphocytic thyroiditis in rats, and that this simple, practical and non-expensive model of experimentally induced thyroiditis could be of importance for further research.

ACKNOWLEDGEMENTS:
This work was supported by Grant No OI-175059 from the Ministry of Science and Technology, Republic of Serbia.

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FENOTIP LIMFOCITA PERIFERNE KRVI U KORELACIJI SA HISTOLOŠKOM SLIKOM ŠTITASTE ŽLEZDE PACOVA TRETIRANIH KALIJUM JODIDOM

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SADRŽAJ

Imajući u vidu ranije rezultate koji su potvrdili povezanost između štitaste žlezde i imunskog sistema, kao i praksu administriranja kalijum jodida u lečenju nekih autoimunih bolesti, smatrali smo da je od naučnog značaja ispitati patogenezu eksperimentalnog tireoiditisa indukovanog kalijum jodidom (KI). Proučavali smo morfologiju timusa, štitaste žlezde, slezine i fenotip T-ćelija (CD4 i CD8) u perifernoj krvi. Eksperimenti su izvedeni na muzjacima Wistar pacova (n=30), nasumično podeljenim u 3 grupe (n=10). Prva grupa je dobila KI (225 μg/g, i.p.), druga grupa je dobila takođe KI (675 μg/g, i.p.), dok je treća grupa bila kontrolna (0.9% NaCl, i.p.). Kalijum jodid je statistički značajno smanjio masu pacova, kao i mase štitastih žlezda ($p<0,05$). On je takođe izazvao visoko statistički značajne ($p<0,01$) histološke promene u vidu mononuklearne ćelijske infiltracije (manja doze KI), destrukcije žlezdanih acinusa i proliferacije vezivnog tkiva (veća doza). Kalijum jodid, sa druge strane, nije uticao na broj CD4 i CD8 T-ćelija u timusu i perifernoj krvi ($p>0,05$). Ovi rezultati ukazuju da KI u određenim dozama indukuje blagi limfocitni tireoiditis, i da ovaj jednostavni i pristupačni model eksperimentalnog tireoiditisa može biti od koristi za dalja istraživanja.