Hormones of Thyroid Gland in Sera of Rats Treated with Different Dose of Concentrated Potassium Iodine Solutions

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SUMMARY

Introduction Potassium iodine (KI) is used as a drug therapy for treating numerous diseases such as small-vessel vasculitis, erythema nodosum, vasculitis nodularis, Sweet's syndrome, tuberculosis and granulomatosis, and for iodized salt. At the same time, KI can be harmful. Iodine intake may increase the frequency of thyroiditis in humans, and may induce the occurrence of experimental thyroiditis (ET) in animals. Investigations on an experimental model for the examination of thyroiditis in Wistar rats have clearly showed morphological changes in the rat thyroid evoked by KI administration.

Objective The purpose of this study was to compare the effects of low and high doses of KI on the thyroid gland of Wistar rats and determine the effect on hormone status (T4, T3 and TSH) in this rat strain.

Methods Two groups of rats from the Wistar strain were treated with a low iodine dose (225 µg/g BW) and with a high iodine dose (675 µg/g BW) of KI solutions. Untreated nonimmunized animals served as controls. The solution was administrated daily intraperitoneally during the period of 26 consecutive days.

Results Monitoring hormone status (TSH, T3 and T4) and morphological changes it was found that therapeutic doses of KI applied in treatment induced the occurrence of experimental thyroiditis (chronic destructive Hashimoto's thyroiditis in humans) and cell necrosis in animals not carrying a genetic susceptibility. Significant inflammatory changes were observed in rats treated with a high iodine dose.

Conclusion The early iodine induced cell necrosis and inflammation in the nonimmunized animals without genetic susceptibility is a new experimental model of thyroiditis.

Keywords: experimental thyroiditis; KI; T3; T4; TSH; rats

INTRODUCTION

Iodine intake may increase the frequency of thyroiditis in humans, and may induce the occurrence of experimental (autoimmune) thyroiditis (ET) in animals [1, 2, 3]. These facts have led to inevitable experimental studies of this serious disease aetiology and pathogenesis. In genetically determined thyroiditis in animals, iodine enrichment has been shown to increase the incidence and severity of the disease. Its mechanism of action is still uncertain [1]. Iodine is believed to be the first known inhibitor of the thyroid gland [4, 5]. In patients with hypothyroidism iodide increases the formation of hormones, and very high doses occasionally inhibit hormone release and lead to gland involution, making the gland harder and reducing blood circulation [1]. In the recent decades increased iodate intake [6] has been shown to enhance significantly the frequency of autoimmune thyroiditis. Iodine effect on the thyroid gland is complex and depends on the dosage and thyroid status of the body [7]. Preoperative administration of Lugol's solution, apart from involution, also causes thyroidite vacuolization and the infiltration of the interstitial space. KI is used as a drug in the treatment of numerous diseases, such as small-vessel vasculitis, erythema nodosum, vasculitis nodularis, Sweet's syndrome, tuberculosis and granulomatosis, and for iodized salt. KI and potassium chloride (KCL) administered to rats stimulate occurrence of thyroid cancer [4]. Radioiodine ablation doses in the treatment of thyroid malignancy provoke permanent thyroiditis as a rule. Iodide induces the release of free radicals in cells and oxidative stress in thyrocytes and possibly in immunocompetent cells [8]. Lithium chloride (LiCl), potassium perchlorate (KClO) and iodides of sodium (NaI) inhibit iodine metabolism in a dose-dependent manner, especially KClO in the dose of 3.5 mmol/l.

Several animal species have been known to be highly susceptible to the development of thyroiditis, as in the case of certain strains of rats and chickens. Large doses of iodide administered to BioBreeding/Worchester BB/W rats, chickens of the Obese strain (OS) and hamsters enhance the incidence of lymphocyte thyroiditis (LT) [9].

The induction of ET has been investigated in genetically susceptible OS with spontaneous development of anti-thyroid antibodies. The thyroid is histologically characterized by mononuclear cell infiltration with the destruction of acini and proliferation of connective tissue, similar to chronic thyroiditis in humans [9]. The administration of iodide to chickens during the first 10 weeks of life increases the incidence of

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ET occurrence, whereas the restriction of iodide decreases the development of anti-thyroid antibodies. This serious inflammation of the thyroid gland is followed by clinical and biochemical features typical of thyroiditis. BB/W rats spontaneously develop insulin dependent diabetes mellitus and LT. Studies have shown that BB/W (Saitama-Tokyo colony) rats develop LT around the tenth week of life. In these rats serum TSH level is increased during LT development, although the level of thyroid hormones remains within the normal range. This means that this rat strain is found to have subclinical hypothyroidism [10].

OBJECTIVE

The purpose of this study was to compare the effects of low and high doses of KI on the thyroid gland of Wistar rats and to determine the effect on hormone status (T4, T3 and TSH) in this rat strain.

METHODS

Animals

Eight to ten week-old male rats of the Wistar strain initially weighing 180-200 g, were used in this study. The study was carried out at the Institute of Pathophysiology of the University of Belgrade. Three groups of 10 rats were treated: the first group was injected with a low iodine dose (LKI – 225 µg/g BW) and the second with a high iodine dose (HKI – 675 µg/g BW) during a period of 26 consecutive days. The control group of animals was injected with saline solution. The rats were injected with specified doses of KI and NaCl daily, and then euthanized by exsanguination from the heart.

Histological analysis

Paraffin sections of the thyroid gland were stained with haematoxylin-eosin or methyl green pyronine. The intensity of histological lesions was classified into four grades from grade 0 to grade 4 as follows: 0 – normal structure; 1 – 1 or 2 foci of infiltration; 2 – 3 or more foci of infiltration; 3 – diffuse infiltration with occasional destruction of follicles; 4 – diffuse infiltration with massive destruction of follicles and proliferation of connective tissue [11].

Quantitative determination of T3, T4 and TSH in animal serum

Serum for hormone assay was obtained from blood samples taken by heart puncture on the 26th day after treatment with solution and kept on -18°C until the determination of hormones.

T3 and T4 concentrations were determined by a radioimmunoassay (RIA) method of the Institute for the Application of Nuclear Energy in Agriculture (INEP Zemun). The RIA of INEP was used to determine total T3 and T4, i.e.the sum of bound and free hormones.

T3 and T4 determination by RIA was based on a competitive binding of hormones in serum and hormones marked as J125 for a certain number of antigen determinants on antibodies specific for these hormones. Thus, radiolabelled and non-radiolabelled immune complexes were developed and precipitated with polyethylene glycol, while free hormones remained liquids. At the same time, the standards of known concentration were treated. After reaction, both complexes were precipitated with polyethylene glycol, while free hormones and free antibodies remained liquids.

The radioactivity of the precipitated complex was measured in a gamma ray scintillation counter. After completed measurement, the mean values of total activity (T), maximal linkage (Bo), and the value of standards and samples (B) were then calculated. The B/Bo ratio expressed as a percentage was calculated according to the formula:

$$B/Bo\ (\%) = CPM/CPM\ (Bo) \times 100;$$
where CPM is the value of standards, samples or control.

Serum total T3 and T4 concentrations could be read directly from the standard curve.

The sensitivity of the assay was 2 nmol/l, which was calculated by exploration of the standard mistake of T4 zero concentration and T3 concentration of 0.027 nmol/l. The reference values of T4 were 55-150 nmol/l. The reference values of T3 were from 1.14-1.40 nmol/l.

Thyreotropin was determined by an immunoradiometric quantitative assay (IRMA). The quantity of radioactivity measured was directly proportional to the TSH concentration of the sample. The normal values were up to 6 mU/1, and the sensitivity of the assay was 0.035 mU/1.

Statistical processing of results

The difference between the experimental groups was analyzed by a one-way ANOVA assay. Intergroup comparison was performed using the Student's t-test.

RESULTS

Histological analysis of the thyroid gland

The thyroid gland of the untreated normal animals is shown in Figure 1a, and the thyroid gland of the control group of the same age treated with saline solution is shown in Figure 1b. No significant differences were found. The cross-section of the thyroid gland of a rat injected with low concentrated KI solution is shown in Figure 1. There were several preserved follicles along its circumference. The remaining portion of the gland was blurred, some remaining follicles without colloid and thyrocytes were observed. Infiltration of mononuclear cells between the gland follicles and proliferation of connective tissue dividing the gland tissue into smaller islands are shown in Figure 1c. All thyroid glands of rats
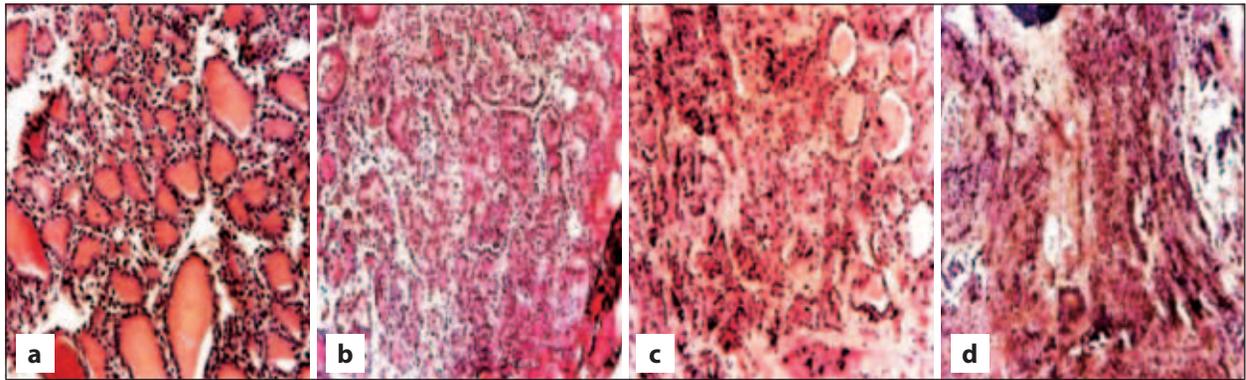
Figure 1. Thyroid glands of the rats: a – nontreated controles; b – controles treated with NaCl; c – experimental group treated with LKI rest of follicules without colloid and thyreocytes, infiltred with mononuclear cells, with proliferation of connective tissue; d – experimental group treated with HKI, with proliferation of connective tissue, lost follicular architecture and mononuclear cell infiltration (H&E, 160×).

Table 1. Intensity of histological changes of the thyroid gland in rats treated with low iodine dose (LKI), high iodine dose (HKI) and controls

Groups	Number	X̄±SD
LKI	10	2.6±2.5
HKI	10	2.5±0.85
Controls	7	0.33±0.52

p<0.01

Table 2. Serum hormone T3 levels (nmol/l) in animals treated with low iodine dose (LKI), high iodine dose (HKI) and controls

Groups	Number	X̄±SD
LKI	10	0.76±0.20
HKI	10	0.80±0.16
Controls	7	0.70±0.14

p<0.05

Table 3. Serum hormone T4 levels (nmol/l) in animals treated with low iodine dose (LKI), high iodine dose (HKI) and controls

Groups	Number	X̄±SD
LKI	10	63.10±16.11
HKI	10	66.30±12.85
Controls	7	88.69±18.98

p<0.01

Table 4. Serum TSH levels (mU/l) in animals treated with low iodine dose (LKI), high iodine dose (HKI) and controls

Groups	Number	X̄±SD
LKI	10	0.17±0.03
HKI	10	0.16±0.03
Controls	9	0.18±0.05

p>0.05

treated with HKI showed changes in terms of connective tissue proliferation, follicle loss and mononuclear cell infiltration, as shown in Figure 1d.

Table 1 presents the results of comparison of the intensity of histological changes in these three groups. The difference between them was statistically significant.

The Kruskal–Wallis analysis of variance revealed a statistically highly significant difference in the intensity of histological changes among the examined groups (H=12.742; p<0.01). Comparing controls and the group treated with LKI by the Mann–Whitney U-test a statistically a highly significant difference was found (U=2; p<0.01), which was also the case with the group treated with HKI (U=1; p<0.01). The test revealed no statistically significant differences in animals treated with KI (U=46; p<0.05).

Hormones

Significant differences in serum T3 hormone levels (nmol/l) between animals of LKI and HKI groups measured at the end of the experiment were analyzed. A statistically significant difference in T3 level between animals of these two subgroups was found (p<0.05). The tests results (t-test) are given in Table 2. One-factor analysis of variance revealed no statistically significant difference in T3 level between the examined groups (F=0.787; p>0.05).

Significant differences in serum hormone T4 level (nmol/l) between animals of LKI and HKI groups measured at the end of the experiment were analyzed. A statistically significant difference in T4 level between animals of these two subgroups was found (p<0.01). The test results (t-test) are given in Table 3. The difference in T4 level between the examined groups was statistically highly significant (F=6.086; p<0.01).

Differences in serum TSH level (mU/l) between animals of LKI and HKI groups measured at the end of the experiment were analyzed. No statistically significant difference in TSH level between the animals of these two subgroups was found; the correlation was negative (p>0.05). The test results (t-test) are given in Table 4. Analysis of variance revealed no statistically significant difference in TSH level between the examined groups (F=0.358; p>0.05).

DISCUSSION

Having in mind that in the recent decades increased iodate intake [6] has been shown to increase the frequency of autoimmune thyroiditis, we analysed the histological changes of the thyroid gland after administration of KI. Animals were treated with different doses of KI solution, and NaCl with [12] or without immunization [12, 13]. In contrast to some other experiments on animals which have not revealed changes

in the gland after administration of mere iodide [14], in our work, in accordance with clinical findings [1, 5, 6, 11] and numerous experiments [3, 8, 9, 10] the gland architecture was seriously damaged. The pathogenesis of follicle destruction and necrosis of thyrocytes were probably induced by a direct toxic effect of iodine on subcellular structures, also suggested by LI Mu [8]. Damages in thyrocytes promote migrations of mononuclear cells into the gland [15], activate complement [12] and provoke inflammatory process since KI has been found to stimulate humoral immunity [12, 16]. After monitoring the hormonal status (TSH, T3 and T4), we found a difference between the treatment and control groups while examining T3 and T4 ratio. The difference in T3 and T4 levels between rat blood treated with LKI and HKI and controls was statistically significant.

The animals euthanized on the 26th day showed a statistically highly significant decrease in T4 level (p<0.01), which is in accordance with our previous study on the effect of minimal dose of KI on the thyroid gland [13], analyzed on the 10th and 17th day. Both cases showed a statistically significant decrease in T3 level (p<0.05). The comparable values of T3 and T4 analyzed on the 10th and 17th day showed no significant differences in the levels of both hormones, when the 17th day was compared with the 10th day. Hypothyreosis increased insignificantly on the 17th day. The exception was the group injected with KI on the 10th day when negative correlation was observed (p>0.05).

Based on our results, T4 level in rats treated with KI showed a significant decrease when compared to the control group level, which is in accordance with other authors' findings [8, 9, 14]. Some authors have reported, however, a decrease of T4 without the incidence of hypothyroidism [5]. The fact, that on the 10th day the decrease of T3 and T4 was observed at daily injection of the specified dose of KI, indicates that this dose could lead to manifest hypothyroidism in blood even quicker, although no statistical difference for T4 was observed [13].

The results obtained by the examination of T3 are in contrast to the results of other authors [17]. Namely, their findings revealed higher T3 levels than ours. They explained it by adaptive mechanisms which enhance peripheral conversion of T4 into T3. Low doses of hormones could be explained by the presence of antithyroid antibodies.

Immunohistochemical methods recorded the presence of antithyroid microsomal antibodies in serum and significantly less titration [18] than one observed in earlier research [13]. However, differences in T3 and T4 levels showed that seven days were not enough to demonstrate more in-depth difference between T3 and T4 levels in blood of already hypothyroid animals.

Iodide effects on the thyroid gland are complex and depend upon the dosage and thyroid status of the body. Preoperative administration of Lugol's solution, apart from the involution, also develops thyrocyte vacuolization [2] and infiltration of the interstitial space. KI and KCL administered orally to rats stimulate the occurrence of the thyroid cancer [4, 19]. Radioiodine ablation doses in the treatment of thyroid malignity cause permanent thyroiditis as a rule [19, 20]. Iodides induce the release of free radicals in cells and oxidative stress in thyrocydes [21] and possibly in immunocompetent cells.

We could agree with the hypothesis on pathogenesis of lymphocyte thyroiditis [11, 22, 23] that environmental factors, iodides, alone or combined with increased TSH level or with a virus may damage the thyroid. The damage induced by iodides associated with hydroxyl radicals is highly severe in sensitive chicken strains [11]. This is followed by the occurrence of numerous phenomena, for example monocytes secrete interleukin 1 which exerts a direct cytotoxic effect on thyrocytes and thereby provide signals to CD4+ cells [2]. T and B lymphocytes migrate into the damaged thyroid, recognize sequestered antigen thyroglobulin as foreign, activate complement and elicit inflammation – thyroiditis [15, 20, 22].

CONCLUSION

Iodide effects on the thyroid gland are complex and depend upon the dosage and thyroid status of the body. By monitoring hormone status (TSH, T3 and T4) and morphological changes it was found that therapeutic doses of KI applied in treatment induce occurrence of experimental thyroiditis (like chronic destructive Hashimoto's thyroiditis in humans) and necrosis of cells in animals not carrying a genetic susceptibility. Significant inflammatory changes were observed in rats treated with HKI.

Early iodine induced cell necrosis and inflammation in the nonimmunized animals without the genetic susceptibility is in fact a new experimental model of the LT.

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Хормони штитасте жлезде у серуму пацова лечених различитим концентрацијама раствора калијум-јодида

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КРАТАК САДРЖАЈ

Увод Калијум-јодид (*KI*) се примењује у лечењу многих болести, као што су васкулитис малих крвних судова, *erythema nodosum*, *vasculitis nodularis*, Свитов (*Sweet*) синдром, туберкулоза и гранулуматоза, као и за јодирање соли. Истовремено *KI* може бити штетан. Уношење јодида код људи повећава учесталост оболевања од тиреоидитиса, а код лабораторијских животиња изазива тзв. експериментални тиреоидитис. Испитивања тиреоидитиса код пацова соја *Wistar* су јасно показала да су морфолошке промене на њиховој штитастој жлезди изазване применом *KI*.

Циљ рада Циљ истраживања је био да се упореде ефекти примене мале и велике дозе *KI* на штитасту жлезду *Wistar* пацова и установи утицај *KI* на њихов хормонски статус (*T3, T4, TSH*).

Методе рада Пацовима соја *Wistar* убризган је *KI* у ниској концентрацији (225 *μg/g* телесне масе) и високој концентрацији (675 *μg/g* телесне масе). Нетретиране неимунизоване животиње служиле су као контрола. Раствор је током 26 узастопних дана свакодневно убризгаван интраперитонеално.

Резултати Мерењем нивоа *TSH*, *T3* и *T4* и посматрањем морфолошких промена штитасте жлезде, установљено је да терапијске дозе *KI* које се примењују за лечење изазивају експериментални тиреоидитис и некрозу ћелија генетски неосетљивих животиња, типа хроничног деструктивног Хашимотовог тиреоидитиса код људи. Запаљенске промене жлезде (инфилтрација мононуклеарних ћелија у разореном ткиву) су биле знатно израженије код пацова који су добијали велике дозе *KI*.

Закључак Некроза и запаљење изазвани јодидом код неимунизованих животиња, без генетске осетљивости, јесте нов и оригиналан експериментални модел тиреоидитиса.

Кључне речи: експериментални тиреоидитис; *KI; T3; T4; TSH*; пацови