Journal of Trace Elements in Medicine and Biology 39 (2017) 210–220

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Biphasic action of iodine in excess at different doses on ovary in adult rats

Dakshayani Mahapatra, Amar K Chandra (Ph.D)*

Endocrinology and Reproductive Physiology Laboratory, Department of Physiology, University of Calcutta, 92, A.P.C Road, Kolkata-700009, India

ARTICLE INFO

Article history:
Received 15 June 2016
Received in revised form 30 August 2016
Accepted 18 October 2016

Keywords:
Excess iodine
Ovary
Female reproduction
Fertility
Steroidogenic enzymes

ABSTRACT

Iodine consumption in excess of its recommended levels over a prolonged period of time is well known to cause thyroid disorders. The thyroid hormones on the other hand are responsible in maintenance of the physiology of the reproductive system. Excess iodine intake affects male reproductive physiology. However, the effects of excess iodine on the ovarian structure and function is yet to be established. The present study has thus been undertaken to investigate the effect of excess iodine on the ovarian physiology. Excess iodine was administered through oral gavage in the form of potassium iodide (KI) for duration of 60 days, at two different doses. The doses used were 100 EI, i.e., 100 times more than the recommended level but tolerable to the thyroid gland and 500 EI, i.e., 500 times more than the recommended level that altered thyroid physiology. The animals were divided into three groups, one control group, and the other two receiving two separate doses (100 EI and 500 EI) of excess KI. Estrous cyclical changes, ovarian morphological changes, ovarian iodine accumulation and ovarian steroidogenic enzyme activities were analysed. The thyroid functional status was studied from the serum thyroid hormones levels. The overall results revealed a biphasic action of excess iodine that depends on its dose. At 100 EI, excess iodine did not alter thyroid physiology but lead to the development of a hypoestrogenic state. There was an increased accumulation of iodine in the ovary with decreased activity of ovarian steroidogenic enzymes and lowered serum estradiol levels. However, at 500 EI, excess iodine developed a hyperthyroid condition, which further leads to a hyperestrogenic state. There was an increased activity of serum steroidogenic enzymes as well as elevated serum estradiol levels. Fertility index was zero in both the 100 EI and 500 EI treated groups of experimental animals. Thus excess iodine (100 EI) ingestion within tolerable range though maintained a euthyroid condition yet developed a state of hypofunctioning ovary. Conversely, excessive iodine (500 EI) is intolerable to thyroid, develops a hyperthyroid condition that leads to a hyperfunctioning ovary. Therefore prolonged exposure of iodine in excess exerts biphasic mode of action depending on the dose in female reproductive physiology and both the doses used in this study affected fertility equally.

1. Introduction

Iodine is one of the most important trace elements required for normal functioning of thyroid gland [1]. Iodine deficiency disorders (IDDs) affect thyroid functions that disturbed other organ systems [2]. Universal salt iodization programme was undertaken to curtail IDDs [3]. However prolonged iodine ingestion in environmentally iodine sufficient areas gradually develops thyroid disorders viz., iodine induced hypothyroidism, hyperthyroidism, endemic goitre, autoimmune thyroid diseases and even cancer of thyroid gland [4–8].

Sodium-iodide symporter (NIS) transports iodide into thyroid follicular cells [9]. Recent evidences have shown varying expression of NIS in the mammalian ovary and uterus through the ovarian

Abbreviations: ANOVA, analysis of variance; BSA, bovine serum albumin; BW, body weight; CL, corpus luteum; DI, diestrous index; E_2, estradiol; E_3, estriol; EDTA, ethylenediaminetetracetic acid; ELISA, enzyme-linked immunosorbent assay; EI, excess iodine; FFC, former follicular cavity; FSH, follicle stimulating hormone; GC, granulosa cells; HSD, hydroxy steroid dehydrogenase; IDDs, iodine deficiency disorders; KI, potassium iodide; LH, luteinizing hormone; NAD, nicotinamide adenine dinucleotide; NaCl, sodium chloride; NIS, sodium-iodide symporter; RIA, radioimmunoassay; SD, standard deviation; SEM, scanning electron microscopy; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; THs, thyroid hormones; TSH, thyroid stimulating hormone; UV, ultraviolet.

* Corresponding author.
E-mail address: physiology.ac@gmail.com (A.K. Chandra).

http://dx.doi.org/10.1016/j.jtemb.2016.10.006
0946-672X/

cycle [10]. Existing literature suggests radioiodide accumulation in ovarian follicles [11] and it has been found to be highest in ovary after thyroid [12]. TSH up-regulates whereas E_2 down-regulates NIS gene expression [13]. The interrelationship between ovarian iodide accumulation and the pituitary-thyroid axis is yet to be affirmed [12]. Altered thyroid physiology also affects ovarian function as well as female fertility [14]. Thus it may be hypothesized that on iodine exposure in excess over a prolonged period of time, the ovarian function would be altered due to its excess accumulation in the ovary and/or for the altered thyroid physiology.

The present study was designed to evaluate the consequences of prolonged exposure of iodine in excess above the recommended and required levels. The study was conducted for 60 days at two different doses [15], one that does not affect the thyroid physiology and serum thyroid hormones (THs) levels and another that altered the thyroid physiology and consequently the serum THs levels. Excess iodine was administered in the form of potassium iodide (KI) orally to adult female albino rats to study the consequent alterations in the morphology and functional status of the ovary with reference to the alterations in the serum THs and iodine nutritional status.

2. Methods and materials

2.1. Reagents

NAD, β-estradiol (98%) and 25% Glutaraldehyde (for Scanning Electron Microscopy) were procured from Sigma, St. Louis, MO, USA. Potassium Iodide (KI), EDTA, Triton X were obtained from Merck, Mumbai, India. ELISA kits were obtained from Institute of Isotopes Co. Ltd. (IZOTOP), Budapest, Hungary. All other reagents were procured from Sisco Research Laboratories (SRL), Mumbai, India.

2.2. Animal maintenance

A total of 120 healthy adult (90 ± 5 days) female albino rats (*Rattus norvegicus*) of Wistar strain weighing 100 ± 10 gm were taken for the study. The animals were divided into three groups (I, II, III), each consisting of 40 animals. The animals were maintained according to the protocol approved by the Institutional Animal Ethics Committee (IEAC), Department of Physiology, University of Calcutta.

The animals were housed in clean polypropylene cages and maintained in a temperature of $22 \pm 2\,^{\circ}C$ and relative humidity of 40-60% in an air-conditioned animal house. A constant 12:12 light:dark cycle was maintained throughout the period of study. The animals were fed on standardized normal diet consisting of 66.5% wheat, 19% Bengal gram, 4.75% fish meal powder, 4.75% dry yeast powder. 0.75% refined til oil, 0.25% shark liver oil, 4% non-iodized salt and water *ad libitum* [16]. Besides this, KI at a dose of 0.007 mg/100 g body weight (BW) was provided to all the animals along with the balanced normal diet [15]. All efforts were made to reduce suffering of the animals.

2.3. Experimental design

The total number of 120 animals were divided into 3 groups (Group I, Group II and Group III), each consisting of 40 animals. Group I was designated as the control (CON) group. Groups II and III were the excess and excessive iodine treated groups at two different doses respectively. All the animals were treated for 60 days. Prior to treatment the estrous cycle of all the animals were studied for at least two consecutive complete cycles, to confirm normal cyclicity of all the experimental animals. The normal duration of the estrous cycle corresponds to 5 ± 1 days [17].

Group I – Group I was the control group, consisting of 40 animals and fed with a normal diet, as mentioned earlier and adequate iodine at the recommended level.

Group II – Excess iodine (100 EI) administered group. This group consisted of 40 animals and were administered excess KI at a dose of 0.7 mg KI/100 g BW dissolved in sterile water and administered by oral gavage. This dose corresponded to 100 times the physiological daily required dose of iodine [15] and henceforth would be referred to as 100 EI (100 times excess iodine). This dose is known not to cause any significant alteration in the thyroid physiology and hence the serum THs levels and thus considered as physiologically tolerable dose [8].

Group III – Excessive iodine (500 EI) administered group. This group consisted of 40 animals and were administered excessive KI at a dose of 3.5 mg KI/100 g BW dissolved in sterile water and administered by oral gavage. This dose corresponded to 500 times the physiological daily required dose of iodine [15] and henceforth would be referred to as 500 EI (500 times excess iodine).

Treatment duration for all the three groups was set at 60 days so as to provide enough time for the animals to undergo a number of estrous cycles that also correspond to an equivalent number of ovarian cycles. The animals were sacrificed 24 h after the last treatment. Animals were grouped and sacrificed according to the stage of estrous cycle (proestrous, estrous, metestrous and diestrous), all animals having the same phase of estrous cycle being sacrificed together. This was done to study the changes in the ovary throughout the different stages of estrous cycle.

Both the doses of excess iodine as used in the form of KI (100 EI and 500 EI) were non-toxic as evident from the serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) levels. The animals were sacrificed by cervical dislocation following protocol and ethical procedures. Blood samples for hormone assay were collected from the hepatic portal vein of rats. Plasma samples were separated by centrifugation at 2000 rpm and stored at $-50\,^{\circ}C$ until assayed.

2.4. Estrous cycle

Estrous cycle for each rat was monitored for at least two complete cycles prior to the initiation of treatment to examine normal cyclicity and ovarian condition of the animals. Then every day from the first day of the treatment till the day of sacrifice estrous cycle of all the animals were studied to assess the changes in the vaginal smear as well as duration of each stage of the cycle in the treated groups compared to the control. For this, normal saline (0.9% NaCl) was taken in a blunt tipped dropper which was put into the vagina of the animal and saline was pushed and the vaginal fluid was instantly sucked out into the dropper. This fluid was smeared on a clean glass slide, stained with eosin and haematoxylin and studied under a bright field microscope to determine the stage of estrous cycle [17].

Diestrous index (DI) was calculated for the entire duration of treatment as follows [18].

$$\text{DI} = (\text{no. of day with clear diestrous}) \times 100/(\text{total duration of treatment})$$

2.5. Body weight and organ weights

The initial body weight (BW) of the experimental animals was recorded on the first day before the treatment and the final BW were recorded on the day of sacrifice. The right ovary of each animal was used for biochemical analysis and the left ovary was subjected to histological and other studies.