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FUNDAMENTAL PRINCIPLES AND TECHNIQUES OF EXPERIMENTAL MODELING OF HYPOTHYROIDISM: A LITERATURE REVIEW

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ABSTRACT — The purpose of our paper is to discuss principles and techniques of experimental modeling of hypothyroidism in laboratory animals, as well as reviewing advantages and disadvantages of experimental models.
Materials and methods. Comparative analysis of contemporary international biological and medical publications in *PubMed/MEDLINE* and *Embase* databases; analysis of contemporary national scientific sources using *Google Scholar* database.
Results. To date, there are six basic principles of experimental modeling of hypothyroidism, i.e.: dietary, drug, surgical, immunological, radioisotope, genetic. Each of the techniques can be used for simulation of the main conditions for hypothyroidism development. Dietary modeling stands for the iodine intake restriction because iodine is an indispensable component in thyroid hormones synthesis. Drug modeling means the use of antithyroid drugs that block thyroid hormones synthesis. Surgical modeling principle involves thyroidectomy. The principle of immunological modeling of hypothyroidism consists in administration of immunosuppressants to animal body. The principle of radioisotope modeling of hypothyroidism lies in acting with a radioactive isotope of iodine on animal body. Genetic modeling principle leads to stimulation of gene mutations in laboratory animals to encode the thyroid hormones formation or their receptors, and results in appearance of the transcription factors responsible for development of the thyroid gland.
Conclusion. Hypothyroidism is a very common pathological condition affecting many organs and tissues. Thus, employment of the hypothyroidism experimental models to study fundamental pathophysiological and pathomorphological processes represents a scientific and research topic of immediate interest. Each of the hypothyroidism modeling principles is specific, and provides for simulation of particular conditions needed for hypothyroidism development in laboratory animals. Taking into consideration numerous beneficial effects of thyroid hormones upon almost all organs and tissues of human body, it is noteworthy that experimental models of hypothyroidism shall be highly sought after by researchers practicing in all medical specialties.

KEYWORDS — experimental simulation technique, hypothyroidism, iodine deficiency, antithyroid drugs, mercazolilum, thyroidectomy, methotrexate, radioactive iodine mutations.

INTRODUCTION

Hypothyroidism represents a very common clinical disorder arising from deficiency of the thyroid hormones, namely the deficiency of triiodothyronine (T3) and tetraiodothyronine (T4). Average occurrence of hypothyroidism in the developed countries is 4–5%, and still in some countries, the hypothyroidism cases occur much more often. For instance, in India, the occurrence of hypothyroidism reaches around 11% [2, 3]. Hypothyroidism has a gender response (it is more common in women), and is age-related (the prevalence of hypothyroidism is higher among older people) [2].

Functional activity of the thyroid gland is inextricably related to the activity of hypothalamus and pituitary gland. In this regard, the organs group into the hypothalamic-pituitary-thyroid system. With involvement of thyrooliberin and thyroid hormones, the hypothalamus and hypophysis activate formation of the thyroid hormone in follicular cells of the thyroid gland. If the level of thyroid hormones in blood is insufficient, the negative mechanism activates adenocytes in anterior pituitary gland. This results in an increase in thyroid hormone (TSH) levels. Its concentration represents one of the most prominent criteria to diagnose hypothyroidism. In addition, TSH levels often increase during the subclinical hypothyroidism, when the clinical manifestations are yet weak or absent and thyroid hormone levels are still within the reference range [35, 40].

Classification of hypothyroidism depends on the hypothalamic-pituitary-thyroid system level at which the disorder occurs. In primary hypothyroidism, the functional activity of the thyroid gland itself is affected and/or impaired. In secondary hypothyroidism, the hypophysis is initially affected, and this leads to the decrease in TSH formation with subsequent inhibition of thyroid function. In tertiary hypothyroidism,

hypothalamus is affected, and this causes inhibition of pituitary adenocytes and follicular thyroid cells.

Besides, hypothyroidism, in relation to the time of its occurrence (development), can be either congenital or acquired. The most common type of hypothyroidism is primary acquired. Its presentation is explained by the insufficient entry of the trace substance of iodine into human body. Hypothyroidism caused by this is particularly representative of the areas endemic for iodine deficiency. In iodine-abundant areas, the main cause of primary acquired hypothyroidism is Hashimoto's disease (autoimmune thyroiditis) [10, 31]. Post-operative and radiation-induced hypothyroidism also act as remarkable prerequisites for primary acquired hypothyroidism [10, 38]. Congenital hypothyroidism is often explained by either the impairment in thyroid development (thyroid dysgenesia), or the impaired thyroid hormone formation (dyshormonogenesis). The root of these disorders lies in gene mutations encoding the formation of transcription factors, receptors and enzymes [8, 46].

Hypothyroidism in its clinical and morphological expressions is quite variable. This is supported by the fact that thyroid hormones target almost all organs and tissues in human body. The manifestation rate of clinical-morphological disorders depends on the thyroid hormone deficiency severity. According to the range of experimental and clinical studies, hypothyroidism produces quite a great effect on skin and its derivatives [1, 43], bone tissue [15, 19], liver [39], as well as the nervous [36, 41] and cardiovascular systems [13, 17].

For the purposes of studying clinical and morphological expressions of hypothyroidism and performing the pre-clinical evaluation of therapeutic and preventive interventions efficiency, the hypothyroidism experimental simulation techniques are often employed. The general scientific research studies, discussing in detail the fundamental principles and methods used to model hypothyroidism, are not available.

The purpose of this research paper is to discuss principles and techniques of experimental modeling of hypothyroidism, as well as reviewing advantages and disadvantages of the existing techniques of hypothyroidism modeling.

BASIC PRINCIPLES OF EXPERIMENTAL MODELING OF HYPOTHYROIDISM

In the best-case scenario, hypothyroidism modeling techniques shall simulate the main conditions for hypothyroidism development. Taking into account the most frequent causes of hypothyroidism development of in a regular practice, it is possible to highlight the following basic principles of hypothyroidism modeling: maintaining the iodine deficient food routine for laboratory animals (dietary model) [30, 45]; thyroidectomy [21, 43], or coagulation of the arteries feeding the thyroid gland without thyroidectomy (surgical model) [24]; oral administration of antithyroid drugs to laboratory animal body (drug model) [6, 20, 25]; stimulation of particular gene mutations responsible for development of the thyroid gland, and biosynthesis of thyroid hormones (genetic model) [22, 23, 32]; radioactive iodine action to laboratory animal (radioisotope model) [37, 44, 47]; and, finally, administration of the immunosuppressive-active drug (immunological model) [26].

Hereafter, this research paper discusses in detail each of these hypothyroidism modeling principles.

DIETARY PRINCIPLE OF HYPOTHYROIDISM MODELING

Despite its little amount in bodies of mammals and humans, trace substance of iodine plays an important role in biosynthesis of T3 and T4. During biosynthesis of the thyroid hormone, molecules of iodine connect with amino acid tyrosine being one of the thyroglobulin protein constituents. In order to form thyroid hormones in sufficient quantity, the daily intake of iodine should average in the range of 100–200 μg. Even with minor reductions in iodine intake (up to 40–80 μg per day), the iodine deficiency condition develops, which, if not leading to clinically significant disorders of thyroid function, causes deviation in the central nervous system development [14, 30]. Besides, subclinical iodine deficiency in food results in the higher risk of atherosclerosis and cardiovascular diseases [16].

Hypothyroidism modeling through the dietary models represent more accurately the true-to-life clinical conditions, as the lack of iodine in food becomes the key factor of primary acquired hypothyroidism development. Various experimental research studies discuss experimental simulations carried out in pregnant rats, and are based on the iodine deficient diet. In laboratory group of animals, the decrease in T3 and T4 biosynthesis and the increase in TSH level of both parents and their newborns is observed. In addition, the cognitive impairment is detected in newborns in laboratory group of animals, as well as the drop in motor activity [45]. M. Kulimbetov et al. proposed the iodine deficient diet based on local produce from the regions of Uzbekistan endemic for iodine deficiency. When modeling hypothyroidism using this diet in rats, the decrease in T4 secretion and structural

rearrangement of the thyroid gland was revealed. This consists in the small-follicular adenomas formation, as well as in the gland weight growth [30].

Primary advantages of the dietary principle of hypothyroidism modeling are gradual decrease in iodine levels and proximity to the true-to-life conditions of clinical practice in view of hypothyroidism development. In addition, the advantages imply that complex invasive and surgical methods are not required.

Among the disadvantages are difficulties in designing specific iodine deficient diets, and the need for constant control and accurate calculation of the iodine concentrations sufficient for hypothyroidism development.

SURGICAL PRINCIPLE OF HYPOTHYROIDISM MODELING

The majority of hypothyroidism surgical models imply complete removal of the thyroid gland (thyroidectomy) in laboratory animals. Thyroidectomy leads to the rapid and persistent drop in thyroid hormone levels [21, 43]. The surgical model enables reproduction of the post-operative hypothyroidism formation which is often observed in clinical practice. Opposite to the dietary model, the surgical simulation of hypothyroidism result in the drop of T3 and T4 concentration much faster. Most often the modeling objects include rats, mice, rabbits, sheep, and dogs.

The research fields involving the surgical models of hypothyroidism vary greatly. For instance, M. Tsujio et al., by means of the surgical models, studied pathomorphological changes in skin, including epidermis and hair follicles, under hypothyroidism. In all rats with thyroidectomy, the slow-down in hair growth was observed 12 weeks after the surgery. Dry and pale skin was also noted, which is representative of hypothyroid condition in humans [43].

M. Helal et al. studied morphological changes in parotid salivary glands in rats under thyroidectomy [21]. In other studies, K. Chen et al. researched in changes in renin-angiotensin-aldosterone system functioning under experimental post-operative hypothyroidism [11, 12]. It is noteworthy, that under hypothyroidism and drop in T3 and T4 levels, the decrease in expression of RNA matrix of the renin enzyme and RAAS inhibition is observed. These changes are associated with the impaired functioning of cardiovascular system, i.e. lowering of heart rate and blood pressure, drop in cardiac output, relaxation of smooth muscle vascular cells, and decrease in total peripheral vascular resistance.

A. Kade et al. proposed another principle of hypothyroidism surgical modeling. It focuses on coagulation of the upper and lower thyroid arteries, which results in thyroid ischemia and drop in T4 and T3 formation. If this model is used, there is no need to dissect the thyroid gland [24].

Among primary advantages of the surgical principle of hypothyroidism modeling are achievement of rapid and persistent hypothyroidism, simulation of conditions needed to develop post-operative hypothyroidism as it is observed in clinical practice.

The disadvantages include the need to involve highly qualified surgical staff to perform operative procedures, complexity of the thyroid glands extraction leading to their thyroidectomy, as well as the decrease in parathyroid hormone formation and drop in calcium levels in blood. Besides, given partial thyroidectomy, development of autoimmune reaction against the remaining part of the thyroid gland is possible. With complete thyroidectomy, parafollicular cells producing calcitonin hormone cease to exist.

DRUG PRINCIPLE OF HYPOTHYROIDISM MODELING

The drug model of hypothyroidism consists in on oral administration to laboratory animals of antithyroid drugs blocking biosynthesis of thyroid hormones [6, 7, 20, 25, 28, 29]. In accordance with the mode of administration and type of antithyroid drug, there exist some varieties of hypothyroidism drug models. Typical antithyroid drugs used in modeling hypothyroidism are mercazolilum (thiamazole, methimazole, tapazole) and propylthiouracil. Their mechanism of action is based on inhibition of thyroid peroxidase enzyme leading to the decrease in active form of iodine formation and the decrease in thyroglobulin iodization. Antithyroid drugs administration is managed through placement of a special gastric tube, or with required dose of the drug dissolved in drinking water [7, 28, 29]. Differences in drug dosing sufficient to achieve hypothyroidism are defined by interspecific differences. For instance, to induce hypothyroidism in rats, appropriate dosing of thiamazole is 2.5 mg per 100 g of animal weight per day for 21 days [25]. To develop hypothyroidism in rabbits, thiamazole dosing of 2 mg per 1 kg of animal weight per day is administered for 21 days [28]. Studies by F. Kamilov proved that the departure from appropriate dosing (2.5 mg/100 g) towards the decrease (1 mg/100 g) did not cause hypothyroidism development in rats, whilst the departure towards the increase (5 mg or more per 100 g of weight) caused multi-organ pathology abnormal for clinical course of hypothyroidism [25].

In another study by Y. Kruk et al., hypothyroidism in rats was simulated by intragastric administration of mercazolilum dosing 10 mg/kg for 2 to 8 weeks. In that case, after 2 weeks, animals developed

mild hypothyroidism; after 4 weeks, its degree was average; and after 8 weeks, it became severe. Along with progressing hypothyroidism, oxidative stress (lipid peroxidation processes) increased in blood and brain tissues in laboratory rats [29].

Since the method of continuous use of the gastric tube causes various types of technical inconvenience for researchers and produces harmful effect on animal body, it was proposed to use hypothyroidism modeling techniques by administering antithyroid drugs with drinking water. The core benefit of this method over intragastric administration is that the drug is continuously administered causing no stress in animals. Thus, the study by H. Bhargava et al. proposed hypothyroidism modeling for 32 days using the drinking water method and containing 0.05% of methimazole. Clinical symptoms (decreased body temperature, decreased systolic blood pressure and heart rate) as well as laboratory data (decreased thyroid hormone levels and increased TSH levels) pointed to hypothyroidism development. In addition, in test group rats, the rate of body weight gain was significantly lower than in control group rats. This attests to important effect of thyroid hormones on metabolic processes [7].

Primary advantages of the drug principle of hypothyroidism modeling are relative simplicity of modeling without any need for qualified surgical staff; broad accessibility and low cost of antithyroid drugs; good solubility of drugs in water. In addition, advantages imply insignificant resources when modeling hypothyroidism in small laboratory animals (rats, rabbits).

The disadvantages of the drug principle of hypothyroidism modeling represent the need to define accurate drug dose necessary for hypothyroidism development. Technical errors are possible; and there is a need for more consistent monitoring of T3, T4 and TSH concentration in blood serum. In addition, placement of gastric tube causes stress to laboratory animals.

IMMUNOLOGICAL PRINCIPLE OF HYPOTHYROIDISM MODELING

In view of the fact that endocrine and immune systems are closely related, action upon the latter can significantly affect thyroid function. Experimental study by S. Kashchenko assessed morphofunctional state of the thyroid gland in laboratory rats under conditions of immunosuppressant (methotrexate) and immunomodulators (immunophan) administration. The authors found out that under intramuscular administration of methotrexate at 50 μg, the evident morphological changes in the thyroid gland occur on the 7th day of the experiment, i.e., deformation of follicles, change in follicular thyrocytes cubic form representative of normothyroidism condition into low-prismatic and flattened being the distinctive evidence of hypothyroidism. In addition, it was revealed that the colloid inside follicles was unevenly distributed, and changed its consistency becoming lumpy and stratiform. Rats in test group also manifested the thyroid weight loss by about 10% on the 7th day if compared to control group. In response to immunomodulators administration, thyroid morphology recovery was observed [26]. Consequently, immune system inhibition goes in parallel with thyroid function inhibition.

Experimental study data are consistent with clinical results. Thus, the scientific sources describe cases of hypothyroidism development in the context of cytostatic methotrexate administration [9]. Therefore, immunological principle of simulating hypothyroidism may be of great interest as regards the enhancement in therapeutic and preventive measures for their subsequent introduction into clinical practice.

Primary advantages of the immunological principle of hypothyroidism modeling are relative simplicity of modeling, intramuscularly drug administration and arrival at high bioavailability.

The disadvantages comprise insufficient research into this model, as well as various side effects triggered by cytostatic drugs.

RADIOISOTOPE PRINCIPLE OF HYPOTHYROIDISM MODELING

The principle of hypothyroidism radioisotope modeling means administration of the radioactive isotope iodine 131I in laboratory animals, which is used in clinical practice to treat hyperthyroidism. Hypothyroidism modeling using radioisotope exposure was mostly carried out in rats and mice. Optimal dosing for animals is 150 μCi that approximately corresponds to the absorbed dose of 0.5 Gy received by the population of the CIS countries at the time of the Chernobyl disaster [44].

V. Usenko et al. completed hypothyroidism simulations in pregnant rats using iodine radioisotopes. At the outcome of simulations, evident changes were found, and were representative of acquired hypothyroidism in both female parents and congenital hypothyroidism in babies of these female rats. T4 level decreased on average by 43%, and through the negative mechanism there was around 8-fold increase in TSH level. The effect of hypothyroidism in female parents on the thyroid gland and nervous system development in fetus depended on the time of exposure to iodine radioisotope. In overall, the newborn rats proved the decrease in body, brain and the thyroid gland weight [44].

C. Reilly et al., simulating hypothyroidism by means of various doses of radioisotope, found that dosing of 50 μCi did not affect much the morpho-functional state of the thyroid gland, whilst dosing of 150 and 450 μCi led to significant drop in thyroid hormone levels and rise in TSH [37]. Radioactive iodine isotope can damage follicular cells that produce calcitonin needed for calcium ion metabolism [5, 42]. Thus, according to the results of experimental studies, after exposure to iodine radioisotope, the number of parafollicular cells in newborns decreases [42], and the number of cells does not recover even 40 days later after exposure to the radioisotope [18]. Taking into consideration this adverse effect of radioisotope on parafollicular cells, it is required to control calcium levels in blood serum.

Primary advantages of the radioisotope principle of hypothyroidism modeling are the achievement of persistent and prolonged hypothyroidism even when employing relatively small amount of iodine radioisotope.

The disadvantages of the radioisotope principle of hypothyroidism modeling represent the need for particular skills to handle radioactive isotopes, as well as negative effects produced by radioisotopes on parafollicular cells.

GENETIC PRINCIPLE OF HYPOTHYROIDISM MODELING

The genetic principle of hypothyroidism modeling consists in stimulation of specific mutations in genes that encode receptors, transcription factors, and enzymes for thyroid hormone biosynthesis. According to the scientific sources, in about 5% of cases hypothyroidism is caused by gene mutations encoding TSH receptor or the following transcription factors: TITF1, FOXE1 or PAX8.

The research group led by E. Amendola [4] designed the genetic model of hypothyroidism. The basic principle of this modeling is to crossbreed heterozygous mice with specific mutations in genes encoding TITF1 and PAX8 factors to produce double heterozygotes. At that, the combination of two heterozygous zero mutations as per TITF1 and PAX8 causes severe hypothyroidism with significant increase in TSH, sharp decrease in thyroid hormone concentration, body weight loss, thyroid hypoplasia, and a higher risk of thyroid hemiagenesis [4]. It was proved, that development of hypothyroidism when using this model stems from the disruption of thyroid organogenesis due to TITF1 and PAX8 deficiency [27, 34].

K. Johnson et al. studied the hypothyroidism specifics in mice with mutations in genes encoding double oxidase enzyme DUOX2 [23] and thyroid peroxidase [22]. Double oxidase is required to form hydrogen peroxide for thyrooperoxidase enzyme, which further catalyzes iodine conversion to its atomic form for inclusion in thyroglobulin protein. Principal clinical and morphological evidence in these mice were pituitary dysplasia, drop in thyroid hormone levels and increase in TSH concentration. In addition, very particular morphological changes associated with hearing impairment in laboratory animals, were impaired formation of internal furrow and cortical tunnel, as well as thickening of tectorial membrane [23].

Snell dwarf mice with mutations in genes encoding pituitary transcription formation of factor Pit1 represent another model of secondary hypothyroidism. This factor is crucial in view of adenocytes development in anterior pituitary lobe, including thyreotropocytes that synthesize TSH [33].

Among primary advantages of the genetic principle of hypothyroidism modeling is the possibility of congenital hypothyroidism mechanisms in-depth studies. Nonetheless, it will not be possible to reconstruct such conditions using other principles of hypothyroidism modeling. This, in fact, makes genetic models unique.

The disadvantages of genetic principle of hypothyroidism modeling imply the use of expensive and not easily accessible equipment needed for the purposes of molecular genetic research.

CONCLUSION

Basing on comparative analysis of the relevant sources, we have identified six basic principles of experimental modeling of hypothyroidism, i.e.: dietary, drug, surgical, immunological, radioisotope, genetic. Each of these principles is specific in its own way, and allows reconstruction of initial hypothyroidism conditions, as well as studying its clinical and morphological expressions. In view of the vast prevalence of hypothyroidism and profound effects of thyroid hormones upon nearly all cells and tissues in mammals, the employment of experimental hypothyroidism modeling can be relevant to doctors from different specialties.

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