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Rat Induced Thyroiditis for Experimental Animal Models: Comparative Study of Caprine Thyroglobulin Injection and Sodium Iodine (NaI) Supplementation

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Abstract: Autoimmune thyroiditis is known to have some causes that need to be observed, including due to the influence of antigens that play a role in the development of this disease, namely thyroglobulin and iodide excess in the body. This study aimed to develop animal models of AITD using injection of thyroid protein goats (*Capra hircus*) and supplementation of Sodium Iodide (NaI). Thyroid protein is known to have a thyroglobulin as major parts of this gland. The development of diseases caused by two distinct inducer will be studied to determine the appropriate methods of AITD detection. Four groups of animal models were used in this study: the control, grup which received supplementation of NaI as well as group which injected with goat thyroglobulin at a dose of 100 and 200 μg mL^{-1}. Histopathological of thyroid gland, serum protein profiles, the expression of IL-1 and thyroxine levels were observed to determine the progression of this disease. Results of this study showed that a dose of 200 μg mL^{-1} thyroglobulin could induce a significant damage to the above parameters and verified the reproducibility. This results was better than induction by NaI supplementation.

Key words: AITD, animal models, caprine thyroglobulin, NaI supplementation, disease

INTRODUCTION

Autoimmune Thyroiditis (AITD) is an organ specific autoimmune disease which attacked the thyroid gland. The main form of this disease are Hashimoto thyroiditis and Graves's disease (Weetman, 2004). This disease are characterized by infiltration of mononuclear cells in the thyroid gland and the production of autoantibodies Thyroglobulin (Tg) and Thyroid Peroxidase (TPO). Patients of AITD are generally suffer from a metabolic disorder caused by dysfunction of the thyroid gland that regulates thyroid hormones due to destruction of the thyroid gland.

Autoimmune thyroiditis affects 5-10% of human population in the world. This occurrence is more common in women than in men with age range between 45-65 years (Akin, 2011). This disease's prevalence rate in 1995-2008 was ranked tobe 2nd most common autoimmune disease appeared in Europe, North America, Australia and New Zealand (Sakaguchi, 2000). Autoimmune thyroiditis has not been reported specifically in Indonesia but it had been reported on 2003, the incidence of thyroiditis disease in Indonesia reached 11.1% of population (Burek and Talor, 2009).

Development of methods for studying pathomechanism, models of therapy and early detection of this disease is through the preparation of animal models of AITD. Some of the methods developed include induction technique, spontaneous and transplantation models. Induction can be done by using a self-antigen. Self-antigen that can be used as an inducer EAT namely Thyroglobulin (TG), Thyroid Stimulating Hormone Receptor (TSH-R) and Thyroid Peroxidase (TPO). Spontaneous models generated by iodide ions due to excess in the body. Sodium Iodide (NaI) with high levels in the body will trigger reactions and toxicity tyrocit as well as autoreactivity of intratiroid proteins that cause AITD.

The amount of thyroglobulin reaches 75% in the thyroid tissue is more likely to succeed than TPO and TSHR autoantigen, besides TG thyroid is an organ specific protein that could significantly induce AITD at EAT (Jin *et al*., 2004; Zhou and Gill, 2005; Song *et al*., 2011). The severity of the resulting TG capable of infiltrating immune cells in the thyroid tissue-specific cellular, i.e., TCD4+cells, TCD8+cells and B cells maximally than TPO and TSHR (Ng *et al*., 2004). Animal models of AITD using TG that has developed from pig Thyroglobulin (pTG), mouse Thyroglobulin (mTG),

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bovine Thyroglobulin (bTG), rodent Thyroglobulin (rTG) and human Thyroglobulin (hTG) (Zhou and Gill, 2005; Song *et al*., 2011; Karras *et al*., 2005; Arata *et al*., 2006). Thyroglobulin from different species can be used in the development of EAT have a similar physical, biochemical and molecular structure so that this study used a goat thyroglobulin or called as caprine Thyroglobulin (cTg).

This research demonstrated the preparation of animal model by comparing the progression of the severity of AITD produced using injection of cTg and NaI supplementation. Histopathological of thyroid, IL-1 expression and the level of Thyroxin (T4) hormone will be reported as well as the protein sera profile by SDS PAGE.

MATERIALS AND METHODS

Animal model: This study use female *Rattus norvegicus* Wistar strain obtained from the Animal Model Unit Development (UPHP) Gadjah Mada University on Yogyakarta with 8-12 weeks of age, body weight between 100-150 g and certified by Brawijaya University Research Ethics Committee No. 148-KEP-UB.

Animals were divided into four groups consisted of 5 rats, the fisrt group was control, second group was gotten supplementation of 0.05% NaI by drinking water (Yu *et al*., 2001; Nagayama *et al*., 2009; Morohoshi *et al*., 2011); the third group was injected by 100 μg mL^{-1} cTg emulsified in Complete Freud's Adjuvant (CFA) and Incomplete Freud's Adjuvant (IFA) as booster and the last group was injected by 200 μg mL^{-1} cTg emulsified in CFA and IFA with the same treatment as previous group. All the treatments were completed during 4 weeks, then rats were sacrified by cervical dislocation.

Data collection and analysis: The observation of thyroid histo section alteration were conducted based on Junqueira (2007) with slight modification. Immunohisto chemistry were performed to analyse the IL-1 expression. Protein in sera analyses were conducted using SDS PAGE and Tyroxin hormone levels were analyses using Rodent T4 Elisa Kit (Endocrintech, USA).

RESULTS AND DISCUSSION

Histopathological observation o thyroid gland of treatment groups is showed as follows:

Figure 1 showed that in normal condition, thyroid gland was composed of irregular follicle wall and lumen containing colloid brightly colored. This condition was different in the thyroid gland AITD groups which showed tissue damage. Group B-D showed the composition of thyroid epithelial cells was irregular and did not surrounded by the thyroid follicles. It also showed mononuclear cell infiltration and destruction of thyroid follicles due NaI administration in rats through drinking water as well as injection of cTg.

Figure 2 performed the Il-1 expressions which were analyzed using axio vission software to perform percentage area. It showed that Il-expressions in rat thyroid tissue has increased compared to the normal rat thyroid tissue. The higher dose of thyroglobulin injection, the greater of IL-1 expression.

The Tyroxin hormone (T4) level of AITD rat in treatment groups was shown on Table 1. The T4 levels of AITD rats induced by thyroglobulin injection were significantly decreased ($p<0.05$). The higher dose of injection, the lower of tyroxin hormone level resulted. Meanwhile, the tyroxin hormone level was increased by induction by NaI supplementation.

Administration of sodium iodide (NaI) were given to rats in excess affects thyroid gland function and thyroid

Table 1: Tyroxin hormone level on treatment groups ($p<0.05$)

Groups	T4 hormone level (ng mL^{-1})
Control	1.722 ± 0.123^{c}
Suplementation of 0.05% NaI	4.4848 ± 0.329^{d}
Injection of cTG 100 μg μL^{-1}	1.472 ± 0.041^{b}
Injection of cTG 200 μg μL^{-1}	1.238 ± 0.119^{a}

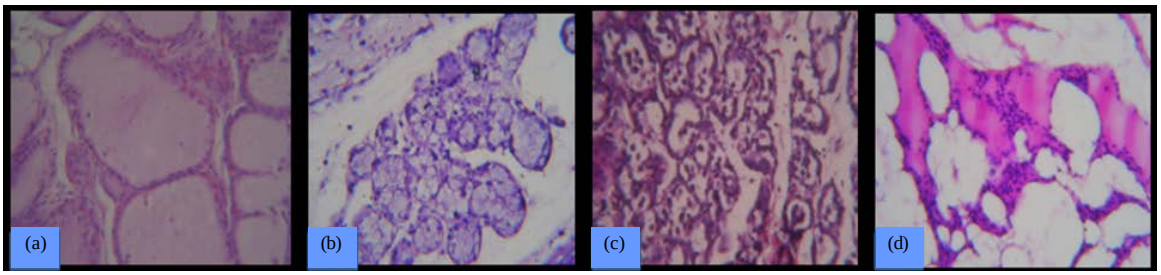

Fig. 1: Histopathological sectioning of thyroid gland (100x); a) control; b) NaI supplementation; c) 100 μg mL^{-1} of cTg and d) 200 μg mL^{-1} of cTg

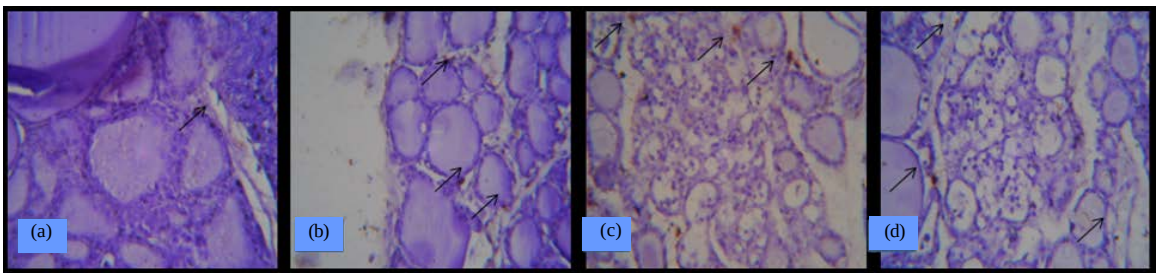

Fig. 2: Il-1 Expression on thyroid gland (100x); a) control; b) NaI supplementation; c) 100 μg mL^{-1} of cTg and d) 200 μg mL^{-1} of cTg

autoimmunity trigger a reaction. Autoimmune reaction characterized by the occurrence of thyroid tissue inflammation that leads to destruction of thyroid tissue. Vary of cTg doses treatment showed significant different of detriment. This result is in line with research of Zhou and Gill (2005) using mTg, Song *et al.* (2011) using the pTg, suggesting that thyroid tissue damage so visible changes in the structure and shape of the follicle. Damaged of rat thyroid tissue characterized by changes in the structure and shape of the follicle caused by the activity of mononuclear cells. However, the presence of mononuclear cells (monocytes) in the rat thyroid was normal in the blood vessels in the thyroid tissue express IL-1 serves to regulate the growth and function thyrocyte. Mononuclear cell infiltration in AITD rats showed monocyte and macrophage cells that attack the follicle cells, it is supported also by the amount of IL-1 expression is increased on AITD group (Fig. 2). This exacerbates the inflammatory reaction and damage to thyroid tissue. Interleukin 1 modulates growth of tyrocyte to perform the function of producing thyroid hormones and modulate apoptotic cells tyrocyte. Chistiakov (2005) mentioned that IL-1 in normal thyroid tissue has function in maintaining the growth and function of tyrocyte. Simons *et al.* (1998) also suggested that IL-1 on normal thyroid condition plays a role to modulate Fas expression induces apoptosis tyrocyte.

Mononuclear cell infiltration in rats AITD thyroid histopathology showed monocyte and macrophage cells that attack the follicle cells, it was supported for increasing IL-1 expression in AITD groups, both due to the NaI supplementation and cTg injection.

Damage was also reported by Bonita *et al.* (2003) which showed infiltration of mononuclear cells and destruction of thyroid follicles due NaI administration in rats through drinking water. Administration of Sodium Iodide (NaI) was given to rats (*Rattus norvegicus*) affected thyroid gland functions and trigger autoimmunity reaction. The increasing of IL-1 expression by NaI supplementation was collateral with the excalation doses of cTg induction. The higher of cTg injection doses given, the higher of IL-1 expression. Antibody (Ab-cTg) then bind and destroy the cTg. Antigen cTg has similarities with Tg on rat thyroid treatment. The Ab-cTg recognize the antigen in rat thyroid. The introduction led to an autoimmune reaction in the rat thyroid. Ab-cTg antibodies then bound to thyroid Tg rats. The bond initiates mononuclear cells (monocytes and macrophages) to express IL-1. Expression of IL-1 initiated the migration of mononuclear cells from blood vessels to the thyroid tissue. The migration caused infiltration of mononuclear cells in the rat thyroid tissue. Infiltration of mononuclear cells resulted in Tg in the follicle lumen and which is still produced in the rat thyroid epithelial cells was digested and destroyed. This mechanism caused damage to the thyroid tissue treated rats. Damage and mononuclear cell infiltration of the thyroid tissue in the treated rats showed a characteristic of AITD. Damaging and infiltration of mononuclear cells in the rat thyroid tissue at the injection EAT thyroglobulin results are also shown by Zhou and Gill (2005), Jin *et al.* (2004), Karras *et al.* (2005) and Arata *et al.* (2006). Tyroxin hormone is a major hormone resulted from thyroid gland. It plays a role in other hormone metabolism. Tyroxine production could be used as AITD diagnose, because in AITD condition, body is not able to control tyroxin hormone production. So, it will be accumulated in blood (Castro and Gourley, 2010). This result was collateral with Piechotta *et al.* (2010) which reported that in NaI excess condition, thyroid gland was not able to maintain thyroxine hormone secretion. In other hand, the higher level of iodine, it will be oxidized by Thyroid Peroxidase (TPO) enzyme resulted in Reactive Oxigen Species (ROS) production as the root of thyrosit cells necrosis.

CONCLUSION

It can be concluded that injection of caprine Tyroglobulin (cTg) could induce AITD in rats models. It resulted in significantly severity and showed better

results than induction by NaI supplementation. This methods was recommended to be used to further study of AITD.

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