A rabbit model of fatal hypothyroidism mimicking “myxedema coma” established by microscopic total thyroidectomy

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Abstract. Myxedema coma (MC) is a life-threatening endocrine crisis caused by severe hypothyroidism. However, validated diagnostic criteria and treatment guidelines for MC have not been established owing to its rarity. Therefore, a valid animal model is required to investigate the pathologic and therapeutic aspects of MC. The aim of the present study was to establish an animal model of MC induced by total thyroidectomy. We utilized 14 male New Zealand White rabbits anesthetized via intramuscular ketamine and xylazine administration. A total of 7 rabbits were completely thyroidectomized under a surgical microscope (thyroidectomized group) and the remainder underwent sham operations (control group). The animals in both groups were monitored without thyroid hormone replacement for 15 weeks. Pulse rate, blood pressure, body temperature, and electrocardiograms (ECG) were recorded and blood samples were taken from the jugular vein immediately prior to the thyroidectomy and 2 and 4 weeks after surgery. The thyroidectomized rabbits showed a marked reduction of serum thyroxine levels at 4 weeks after the surgical procedure vs. controls (0.50±0.10 vs. 3.32±0.68 µg/dL, \(p<0.001\)). Additionally, thyroidectomized rabbits exhibited several signs of hypothyroidism such as hypothermia, systolic hypotension, bradycardia, and low voltage on ECGs, compared with controls. Of the 7 rabbits with severe hypothyroidism, 6 died from 4 to 14 weeks after the thyroidectomy possibly owing to heart failure, because histopathologic examinations revealed a myxedema heart. In summary, we have established a rabbit model of fatal hypothyroidism mimicking MC, which may facilitate pathophysiological and molecular investigations of MC and evaluations of new therapeutic interventions.

Key words: Myxedema coma, Animal model, Hypothyroidism, Total thyroidectomy, Thyroid hormone

Myxedema coma (MC) features severe and life-threatening hypothyroidism with high mortality rates variously reported as 25% (2 of 8 patients) [1], 36% (4 of 11) [2], and 52% (12 of 23) of affected individuals [3]. Because of its rarity and the sudden presentation of hypothyroidism, which make it unfeasible to conduct a randomized control trial, there are no general guidelines for the diagnosis and treatment of MC.

Currently, the most common approach to treat MC is the replacement of thyroid hormone [4-7]. However, an optimal method for thyroid hormone replacement therapy for MC has not yet been established [4-7]. Therefore, a valid animal model is required to explore new therapeutic interventions.

Although there have been several reports regarding a rabbit model of thyroidectomy [8-10], no study has focused on the establishment of MC and simultaneously suggested the analysis of MC pathogenesis and treatment. In one study [8], for example, serum thyroxine values were shown to be reduced to 23% of the normal range at 6 to 8 weeks after total thyroidectomy. These thyroidectomized rabbits also exhibited several signs of hypothyroidism such as hypothermia, coarse
hair, and the development of fat deposits throughout the body; however, they did not develop MC [8].

To address this issue and provide a platform for the study of MC pathogenesis and treatments, the aim of the present research was to establish a rabbit model of MC induced by total thyroidectomy performed under a surgical microscope.

Materials and Methods

Animal study design

Ethical issues

All research animals were treated in accordance with the Principles of Laboratory Animal Care formulated by the U.S. National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institute of Health. The research protocol was approved by the Animal Research Committee of the National Defense Medical College, Tokorozawa, Japan.

Animal preparation

Male New Zealand White rabbits (specific pathogen-free) weighing 2.5 to 3.0 kg were obtained from a commercial supplier (Japan SLC, Inc., Shizuoka, Japan). The rabbits were housed individually in stainless steel cages (height 35 cm, width 50 cm, and depth 55 cm) and provided commercially available standard rabbit pellets (CLEA Rabbit Diet CR-3; CLEA Japan, Inc., Tokyo, Japan) and tap water at all times. The room was kept at a constant temperature (25°C) and light/dark cycle (lights on from 0500 to 1900 h). The rabbits had at least 5 days before surgery to become acclimated to the new rhythm and environment. Animals were randomly divided into either a control (sham operation) group (n = 7) or a thyroidectomized group (n = 7).

Physiologic examination and blood sampling

In this experiment, physiologic examinations and blood samplings were performed three times in both groups; just before the thyroidectomy or sham operation (i.e., day 0), on day 14, and on day 28.

Rabbits in each group were weighed and anesthetized via intramuscular injection of 35 mg/kg ketamine (Sankyo, Tokyo, Japan) and 5 mg/kg xylazine (Bayer Yakuhin, Ltd., Tokyo, Japan) administered using a 23-gauge injection needle [11, 12]. Following disappearance of the hind-limb pedal reflex, the rabbits were placed on an operating table in the supine position. The ambient temperature was maintained at 24–26°C during the procedure. An electronic thermometer probe (KN-91 for rabbits; Natsume Seisakusho, Tokyo, Japan) was inserted rectally for continuous monitoring of the body core temperature. The blood pressure in the calf was measured using a noninvasive blood pressure monitor and a 25-mm-wide cuff (SurgiVet V6004; Smiths Medical PM, Inc., Waukesha, WI, USA). The cuff was wrapped around the calf along the course of the posterior tibial artery to determine the pulse rate and the systolic and diastolic blood pressures [13, 14]. To evaluate the heart rate and cardiac rhythm by electrocardiogram (ECG) (Life Scope BSM-5192; Nihon Kohden Co., Tokyo, Japan), three needle-type ECG electrodes were inserted into the front limbs and left pelvic leg of the subject within the subcutaneous area. The ECGs were recorded as a lead-II system (sensitivity ×8). Blood samples (10 mL) were taken from the jugular veins using an 18-gauge catheter and a 10 mL syringe. We administered 10 mL saline solution (0.9%) (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) intravenously to replace the blood volume with fluids. After the needle was withdrawn, we applied gentle pressure with a cotton ball to the puncture site until the bleeding stopped.

Thyroidectomy procedure

After the induction of anesthesia, the operation site was shaved and disinfected in both groups. An approximate 4-cm long incision was made with a scalpel in the skin at the midline axis of the neck. The wound was dilated gradually with blunt forceps to expose the thyroid gland and the muscles and connective tissue were separated therefrom. In the thyroidectomized group, under a surgical microscope (OPMI pico i; Carl Zeiss Inc, Oberkochen, Germany), both thyroid lobes along with the isthmus were dissected free from the trachea and cricoid and removed completely using a bipolar electrosurgical forceps (straight, tip 1 mm, blunt, length 145 mm, ERBE No. 20195-038; AMCO Inc. Tokyo, Japan) with an electrosurgical generator (EndoCut mode 60 W, ERBE ICC200; AMCO Inc.). In the control (sham operation) group, the exposed thyroid gland was not dissected. The wound was carefully swabbed, and the skin was then sutured and knotted securely. The surgical procedure was performed under sterile conditions and completed within 30 min following anesthesia initiation. No antibiotics were administered after the surgical procedure because this often results in the occurrence of severe enterocolitis in rabbits [15].

Post-operative observational course

The rabbits in each group were monitored without
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...thyroid hormone replacement for 15 weeks. When death occurred during the observational period, tissue samples were taken from the right ventricles, lung, kidney, and liver to clarify the cause of death. At the end of the study, all animals were sacrificed according to the instructions given by the Institutional Animal Care and Use Committee of the National Defense Medical College.

**Results**

All rabbits in both groups were allowed to recover from anesthesia in their cages on days 0, 14, and 28. No symptoms of tetany or infection were observed after surgery.

Images of the total thyroidectomy and thyroid grand histology are presented in Fig. 1. We confirmed that the removed organs were thyroids without pathologic changes.

The vital signs and laboratory findings of the rabbits are shown in Table 1. The animal model in the present study showed a marked reduction of serum TT3 and TT4 levels at 2 and 4 weeks (i.e., days 14 and 28, respectively) after total thyroidectomy. Additionally, compared with controls, the thyroidectomized rabbits exhibited several signs of hypothyroidism such as hypothermia, systolic hypotension, bradycardia, anemia, renal dysfunction, and dyslipidemia (a rise in the serum total cholesterol value). All thyroidectomized rabbits showed jugular venous distention at the time of blood sampling on days 14 and 28.

In the thyroidectomy group, 6 of 7 rabbits died between 4 and 14 weeks after total thyroidectomy, on days 31, 39, 43, 52, 94, and 101, respectively. No rabbit in the control group died during the observational period. The Kaplan-Meier plots of survival after the operation generated to illustrate the difference between the control and thyroidectomized groups are presented in Fig. 3. The survival rate was higher in the control group than in the thyroidectomized group ($p = 0.001$).

Fig. 4 shows the representative histology of the heart, lung, liver, and kidney of autopsied rabbits in the thyroidectomy group. In the right heart ventricle, interstitial edema and thin myocardium were observed. Myocardial cells had a perinuclear halo, and some had...
Fig. 1 Images of total thyroidectomy and thyroid gland histology. (A) The two reddish lobes on the upper trachea connected by the isthmus are the thyroid glands of a rabbit. (B) Total thyroidectomy procedure for a rabbit. (C) Gross anatomical appearance of the thyroid glands derived from a rabbit. (D) Gross anatomical appearance of the trachea after total thyroidectomy. (E) Thyroid gland histology (hematoxylin-eosin). No pathologic changes were observed. The scale bar indicates 500 μm.
**Table 1** Vital signs and laboratory findings in rabbits on days 0, 14, and 28

|                      | Day 0 (Pre-surgery) | Day 14 (Post-surgery) | Day 28 (Post-surgery) |
|----------------------|---------------------|------------------------|-----------------------|
|                      | All rabbits (n = 14) | Controls (n = 7)       | Thyroidectomized (n = 7) | p value |
|                      |                     | Controls (n = 7)       | Thyroidectomized (n = 7) | p value |
| Body weight (kg)     | 2.9 ± 0.1           | 3.29 ± 0.20            | 3.0 ± 0.3              | 0.029 |
|                      |                     | 3.41 ± 0.23            | 2.9 ± 0.3              | 0.001 |
| Systolic BP (mmHg)   | 116 ± 12            | 121 ± 15               | 92 ± 12                | 0.002 |
|                      |                     | 117 ± 15               | 84 ± 16                | 0.000 |
| Diastolic BP (mmHg)  | 69 ± 25             | 58 ± 18                | 50 ± 9                 | 0.32  |
|                      |                     | 58 ± 10                | 49 ± 9                 | 0.12  |
| Pulse rate (/min)    | 219 ± 33            | 235 ± 23               | 154 ± 17               | <0.001 |
|                      |                     | 212 ± 22               | 141 ± 20               | <0.001 |
| Body temperature (°C)| 40.2 ± 0.3          | 40.0 ± 0.1             | 39.1 ± 0.2             | <0.001 |
|                      |                     | 40.1 ± 0.4             | 38.5 ± 0.3             | <0.001 |
| White blood cells (/μL)| 4,400 ± 900        | 4,500 ± 1,100          | 6,100 ± 1,400          | 0.033 |
|                      |                     | 4,100 ± 900            | 5,300 ± 1,400          | 0.092 |
| Hemoglobin (g/dL)    | 11.7 ± 0.8          | 12.3 ± 0.8             | 11.0 ± 0.6             | 0.003 |
|                      |                     | 12.6 ± 0.5             | 10.4 ± 0.5             | <0.001 |
| Platelets (×10^9/μL)| 25.2 ± 6.3          | 25.8 ± 7.5             | 26.5 ± 3.8             | 0.84  |
|                      |                     | 24.1 ± 7.3             | 23.8 ± 4.1             | 0.91  |
| Sodium (mEq/L)       | 142 ± 4             | 141 ± 2                | 138 ± 3                | 0.07  |
|                      |                     | 141 ± 4                | 138 ± 4                | 0.26  |
| Potassium (mEq/L)    | 3.7 ± 0.3           | 3.8 ± 0.2              | 4.1 ± 0.3              | 0.044 |
|                      |                     | 3.6 ± 0.4              | 4.0 ± 0.4              | 0.094 |
| Chloride (mEq/L)     | 96 ± 8              | 102 ± 3                | 103 ± 3                | 0.41  |
|                      |                     | 94 ± 8                 | 102 ± 3                | 0.026 |
| Calcium (mg/dL)      | 11.4 ± 1.7          | 13.5 ± 0.5             | 10.2 ± 1.0             | <0.001 |
|                      |                     | 10.3 ± 2.0             | 9.8 ± 1.7              | 0.58  |
| Phosphorus (mg/dL)   | 4.8 ± 1.1           | 5.1 ± 0.8              | 3.9 ± 0.6              | 0.01  |
|                      |                     | 3.8 ± 0.6              | 4.4 ± 0.9              | 0.13  |
| AST (U/L)            | 0.3 ± 0.1           | 0.4 ± 0.1              | 0.3 ± 0.1              | 0.12  |
|                      |                     | 0.3 ± 0.1              | 0.3 ± 0.1              | 0.21  |
| ALT (U/L)            | 31 ± 30             | 35 ± 19                | 35 ± 22                | 0.99  |
|                      |                     | 28 ± 16                | 44 ± 36                | 0.29  |
| ALP (U/L)            | 356 ± 92            | 331 ± 74               | 404 ± 254              | 0.48  |
|                      |                     | 261 ± 53               | 481 ± 580              | 0.34  |
| LDH (U/L)            | 331 ± 194           | 339 ± 154              | 209 ± 68               | 0.065 |
|                      |                     | 196 ± 44               | 208 ± 76               | 0.71  |
| Albumin (g/dL)       | 4.3 ± 0.7           | 5.1 ± 0.5              | 3.5 ± 0.5              | <0.001 |
|                      |                     | 3.6 ± 0.9              | 3.8 ± 0.5              | 0.54  |
| BUN (mg/dL)          | 11.8 ± 2.8          | 15.9 ± 2.2             | 24.0 ± 5.0             | 0.002 |
|                      |                     | 13.3 ± 3.2             | 31.7 ± 2.8             | <0.001 |
| Creatinin (mg/dL)    | 0.6 ± 0.1           | 0.7 ± 0.1              | 1.0 ± 0.2              | 0.002 |
|                      |                     | 0.5 ± 0.1              | 1.5 ± 0.2              | <0.001 |
| Glucose (mg/dL)      | 168 ± 43            | 146 ± 26               | 122 ± 11               | 0.046 |
|                      |                     | 127 ± 25               | 123 ± 21               | 0.74  |
| T-Chol (mg/dL)       | 19 ± 7              | 18 ± 6                 | 81 ± 45                | 0.003 |
|                      |                     | 12 ± 5                 | 131 ± 77               | 0.001 |
| CK (U/L)             | 2,097 ± 1,130       | 2,300 ± 1,077          | 1,892 ± 473            | 0.38  |
|                      |                     | 1,305 ± 762            | 1,838 ± 788            | 0.22  |
| TT3 (ng/mL)          | 1.05 ± 0.25         | 0.94 ± 0.07            | 0.22 ± 0.04            | <0.001 |
|                      |                     | 0.84 ± 0.10            | 0.22 ± 0.03            | <0.001 |
| TT4 (μg/dL)          | 3.95 ± 0.83         | 3.71 ± 0.84            | 0.54 ± 0.13            | <0.001 |
|                      |                     | 3.32 ± 0.68            | 0.50 ± 0.10            | <0.001 |

Data are shown as the means ± SD. All p values are from Student’s t-tests. BP, blood pressure; T-Bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; T-Chol, total cholesterol; CK, creatine phosphokinase; TT3, total triiodothyronine; TT4, total thyroxine.

**Fig. 2** Electrocardiogram data (lead-II, sensitivity ×8) from a rabbit prior to the surgical procedure on day 0 (A), from a control rabbit on day 14 (B) and on day 28 (C), and from a thyroidectomized rabbit on day 14 (D) and on day 28 (E).
branches. These findings in the heart were compatible with the myxedema heart [16, 17]. In addition, lung histology revealed congestion and edema, suggestive of left heart failure. Congestion of both the liver and kidneys also suggested the presence of right heart failure.

**Discussion**

In this study, we demonstrate the establishment of a rabbit model of fatal hypothyroidism mimicking MC that was induced by microscopic total thyroidectomy. MC in humans is defined as a life-threatening endocrine crisis induced by severe hypothyroidism, which exhibits hypothermia, circulatory failure, respiratory failure, and hyponatremia, finally leading to an altered mental status. The present rabbit model similarly presented with hypothermia and circulatory failure resulting from severe hypothyroidism, followed by death in most cases. Furthermore, this animal model with severe hypothyroidism also exhibited several signs of heart failure such as distention of the jugular veins, low voltage on ECG, systolic hypotension, bradycardia, and histopathologic findings of congestive heart failure owing to a myxedema heart. Together, the results indicated that the thyroidectomized rabbits with severe hypothyroidism died of congestive heart failure.

The distinctive feature of the present study compared with previous reports [8-10] was that we performed a total thyroidectomy for rabbits under a surgical microscope, which was developed following technological progress. We speculate that the researchers in previous reports [8-10] performed the surgery for total thyroidectomy on rabbits without the use of surgical microscopes. Consequently, the rabbits did not develop MC because the thyroid glands of the rabbits might not have been completely removed.

We consider the present model has several advantages over previous hypothyroid models that utilized methimazole or propylthiouracil (PTU) [18-21]. These drug-induced models have been traditionally used because of their simplicity [19-21]; however, inconsistencies in drug absorption might occur in these models owing to repeated oral administration. Furthermore, PTU-induced models have demonstrated a reduction of serum TT3 and TT4 levels [19], dyslipidemia [20], and mild bradycardia (188±16 heart beats/min) [21], but have not exhibited features of severe hypothyroidism including hypotension, anemia, renal dysfunction, and MC. In contrast, the present model demonstrated comprehensive manifestations of severe hypothyroidism with eventual lethality owing to myxedema heart. In addition, antithyroid drugs possess certain drawbacks such as clinically important immunosuppressive effects and potentially life-threatening side effects, *i.e.*, agranulocytosis, hepatotoxicity, and antineutrophil cytoplasmic antibody-positive vasculitis [22]. PTU can also form reactive intermediates that promote autoimmune inflammation [22]. Together, these effects are disadvantageous in establishing animal models of hypothyroidism. Finally, the present model, compared with PTU-induced hypothyroid models exhibiting less complete ablation and concomitant side-effects, will likely be more useful in future research to develop regenerative therapies for hypothyroidism.

In establishing a hypothyroid model induced by total thyroidectomy, the possibility of the presence of ectopic thyroid in rabbits must also be considered. However, we were unable to find any published evidence regarding the incidence of ectopic thyroid in rabbits. Notably, in a study that identified both thyroid and parathyroid tissues via near infrared fluorescence imaging following intravenous methylene blue administration, no soft tissues were identified in the rabbit neck central compartment excluding the orthotopic thyroid and parathyroid glands [23]. Furthermore,
Autopsy findings in the thyroidectomized rabbits dying of heart failure owing to a myxedema heart. (A) Right heart ventricle: Interstitial edema and thin myocardial cells can be observed. (B) Right heart ventricle: Within the edematous interstitium, myocardial cells exhibit a perinuclear halo and some display branching features. (C) Lung: Capillaries within the alveolar wall are filled with red blood cells and fluids can be observed within the alveoli. (D) Liver: Centrilobular congestion can be observed. (E) Kidney: Capillaries within the glomerulus and interstitium are filled with red blood cells. (A-E): hematoxylin-eosin; the scale bars indicate 500 µm in (A), 50 µm in (B), and 100 µm in (C-E).
the demonstration by another study of the distribution of radioactive iodine in rabbit tissues after intravenous radioisotope (Iodine-130) administration indicated that the radioiodine concentration in the thyroid was at least one hundred times higher than that in other tissues including the heart, lung, and blood [24]. These findings can be interpreted to mean that ectopic thyroid tissue likely did not exist within the main organs. Together, these reports indirectly suggest that the potential effect of ectopic thyroid would be subtle in our model, if such tissue was present at all. Finally, because residual thyroid function would be reflected in the monitored values, the reliability of the present model can be supported with the use of adequate postoperative monitoring.

There are several reasons to select rabbits for establishing an animal model of MC. Rabbits have four parathyroid glands, two within the thyroid and two in close proximity to the carotid sheath outside of the thyroid, in comparison with other species whose parathyroid glands are all embedded within the thyroid [8, 23, 25]. Consequently, the calcium homeostasis of the rabbit is known to be stable after total thyroidectomy without calcium and/or vitamin D supplementation because of the existence of the two parathyroid glands outside the thyroid [8, 23, 25]. Furthermore, owing to their relatively larger body-size compared with rats or mice, it is easier to perform repeated blood sampling, surgical procedures, and treatment experiments on rabbits.

Investigation of the cause of death in the thyroidectomized rabbits indicated that these animals died of congestive heart failure owing to a myxedema heart. We excluded infection as a cause of death because of both the absence of leukocytosis after surgical procedures and inflammation on autopsy. We also excluded the possibility of tetany because of the absence of hypocalcemia. Furthermore, we excluded bleeding and/or anesthesia as causes of death because no rabbits died on the day of the surgical procedure. The thyroidectomized rabbits did, however, exhibit several signs of hypothyroidism such as hypothermia [26], anemia [27, 28], renal dysfunction [29-31], and dyslipidemia [32]. A previous study showed that PTU-induced hypothyroidism in rats led to severe systolic dysfunction and heart failure [33]. In our experiments, the rabbits with severe hypothyroidism showed distention of the jugular veins, systolic hypotension, bradycardia, and low voltage on ECG. Additionally, histopathologic examinations revealed a myxedema heart presenting with interstitial edema, thin myocardial cells with perinuclear halo, and branching [16-17]. Furthermore, we confirmed congestion of the lung, liver, and kidney along with the congestive heart failure.

Blood sampling is required to investigate biomarkers in an animal model with hypothyroidism. Although rabbit ear vessels are easily accessible, vascular spasm caused by needle puncture often makes it difficult to take blood samples [34]. Additionally, repeated blood sampling from the ear vessels might result in hemolysis or insufficient sample volume [35]. In the present study, sufficient sample volume and frequent blood sampling were needed for biomarker analysis. Therefore, we opted to take blood samples from the jugular veins instead of from ear vessels to improve the accuracy of the biomarker values.

In our study, the removal of 10 mL blood from the rabbits (weighing 2.5–3.0 kg) represented approximately 6–7% of their total blood volume [36]. To compensate for this, we replaced the same volume (10 mL) using normal saline [37]. Single blood sampling below 7.5% of the circulatory blood volume takes approximately 1 week to recover [36]. Therefore, we took blood samples 2 weeks after each prior blood sampling. We considered that the anemia observed in the thyroidectomized rabbits on days 14 and 28 was not due to blood sampling because the control animals, from which the same volume of blood had been taken, did not exhibit anemia. We further considered that the systolic hypotension in the thyroidectomized rabbits observed on days 14 and 28 was also not due to blood sampling because a previous report showed that hypovolemic shock could be caused by a single blood sampling beyond 15% of the circulatory blood volume [36]. Therefore, we believe that the blood sampling volume taken in our study had little effect on the validity of the data.

The present study has some limitations. First, we did not perform postoperative radioactive iodine imaging to confirm the presence of residual or ectopic thyroid tissue. However, if present, such tissue would be noted by monitoring during the postoperative observation course and by a failure of MC-induction. Second, we were unable to evaluate the effects of potential stress response to the anesthetics and blood sampling on our data. Third, at least 4 weeks are required after total thyroidectomy to induce the present rabbit model.

In conclusion, we have established a rabbit model of fatal hypothyroidism mimicking MC that is induced by microscopic total thyroidectomy. This model exhib-
mented several signs of severe hypothyroidism 2 weeks after total thyroidectomy and fatality between 4 and 14 weeks. In these animals, the decreased contractile activity of the hypothyroid heart might have caused congestive heart failure, resulting in death. Our animal model will likely be useful to facilitate the pathophysiological and molecular investigation of total hypothyroidism, serve as a platform for the development of new treatments, therapies, and biomarkers, and establish optimal thyroid hormone replacement therapy protocols for MC.

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Disclosure

No competing financial interests exist.

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