

—Originals—

The influence of hypothyroidism on wound healing

An experimental study

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Abstract

An experimental study was performed to investigate the influence of hypothyroidism on wound healing. A state of severe hypothyroidism was induced initially by performing a total thyroidectomy on rat models; subsequently wounds were made by making long midline abdominal incisions and then suturing them. The parameters used to evaluate the process of wound healing of these incisions were the assay of type-I collagen, type-III collagen (procollagen peptide PIPC and P III P, each being the precursor of collagen), type-IV collagen, and hydroxyproline. The assays were repeated at specific times and compared to assays of similar parameters taken from a control group. In the state of hypothyroidism, a decrease was observed in type-IV collagen and hydroxyproline during the proliferative phase of wound healing. This indicated that the state of hypothyroidism constitutes an important factor in delaying wound healing.

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Key words: hypothyroidism, wound healing, hydroxyproline, collagen

Introduction

Delay in wound healing has been observed in the postoperative period of patients who are in a severe hypothyroid state as a result of total thyroidectomy or radiotherapy to the neck¹⁻⁴. On the other hand, there have been clinical reports⁵ of keloid development during the healing process of wounds of patients with hyperthyroidism. Basic research on the influence of abnormal thyroid function on wound healing remains scarce and has not yet been clearly established.

In order to analyze the relationship between hypothyroidism and wound healing, we induced a state of severe hypothyroidism in rats by surgically resecting their thyroid glands, and then assaying the levels of hydroxyproline and procollagen peptide (type I and III), which are the precursors of collagen and collagen (type IV: 7 S) in wounds.

Materials and Methods

Wistar-strain male rats, 12 weeks old and fed with solid ordinary foodstuff (F-2 Sankyo Labo Service), were used in the experiments. These rats were divided into two groups; a surgical hypothyroidism rat group (hereafter referred to as the SHT-group) and a control group. Rats in the SHT-group were anesthetized with ether and then a 1.5 cm skin incision was made in the cervical midline. The thyroid glands were then almost totally removed (**Fig. 1**). Similar skin incisions were made in the rats in the control group; however, in contrast to the rats in the SHT-group, their thyroid glands were left intact. Since functional recovery of the remnant thyroid in the SHT-group rats may occur at about 8 – 10 weeks⁶, the rats were used for experiments 10- weeks or longer from the date of the thyroidectomy.

Firstly, the thyroid status of each rat was evaluated

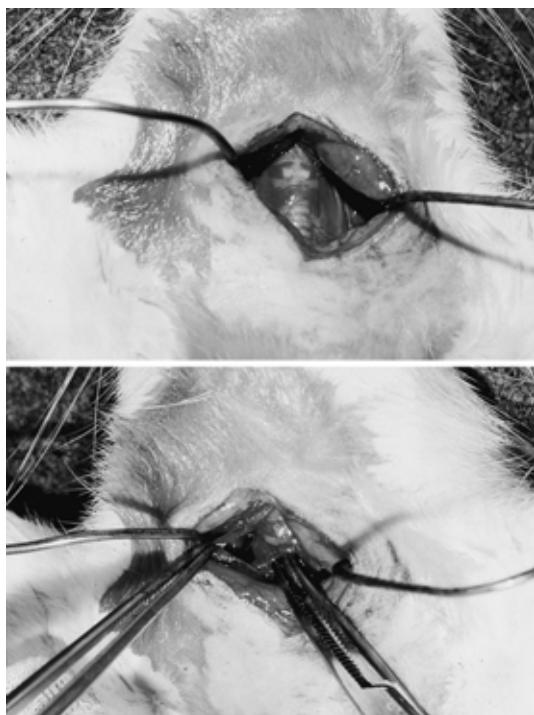


Fig. 1 Surgical view of total thyroidectomy

by measuring the serum thyroid stimulating hormone (TSH) level. This was carried out by drawing a 1.5 ml sample of blood from the femoral artery using a 2.5 ml syringe and 26 G needle (Terumo Corporation). A midline incision of more than 5 cm was then performed over the abdomen extending from the xiphisternum downwards and deepened until just above the fascia. The incised wound was subsequently sutured with a 5-0 nylon suture over a width of 5 mm and at intervals of 5 mm around the wound. This was in view of the fact that the biochemical active zone⁷ occurs within the range of 5 mm on both sides of the wound edges where most parts of the change in the matrixes and collagen of wound healing take place. On the 1st, 3rd, 7th, and 14th postoperative day, a 10 mm wide skin sample was removed from the wound of each rat (Fig. 2). Hydroxyproline and procollagen peptide (PIPC, P III P), which are the precursors of collagen, and type IV collagen in the resected skin were assayed.

Rat Thyroid Stimulating Hormone Radioimmunoassay (rTSH RIA) (National Institute of Diabetes and Digestive and Kidney Diseases) was used for TSH assay. Type-I collagen (type-I procollagen c-terminal peptide, PIPC) and type-III collagen (type-III procollagen N-terminal peptide, P III P) were measured by radioimmunoassay. 7S content was also measured for type-IV collagen assay in virtue of radioimmunoassay.

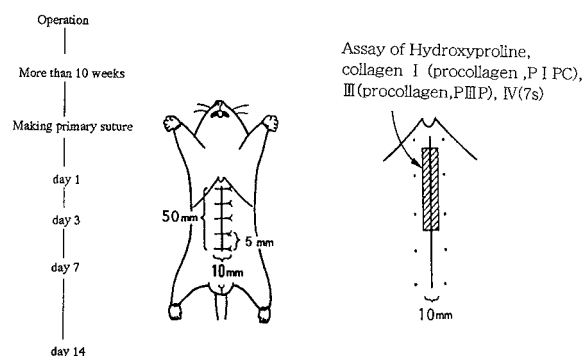


Fig. 2 Rat experimental model for evaluation of Hydroxyproline and collagen in skin tissue

Hydroxyproline was assayed by the High Performance Liquid Chromatography (HPLC) method. The student-T test was used for statistical analysis.

Results

1. Thyroid function in the SHT and control groups

A statistically significant difference was noted in the serum TSH level between the SHT and control groups at the time of wound development. Overt hypothyroidism was confirmed in the SHT-group (Fig. 3).

2. Collagen

(1) Procollagen peptide

This procollagen peptide is a peptide that is bound to both ends of the collagen

molecule isolated from the procollagen peptidase when procollagen, the precursor of collagen, is synthesized intracellularly, and secreted extracellularly, and polymerizes as a collagen molecule. PIPC and P III P reflect the amount of type-I collagen and the type - III collagen, respectively.

Because of the large deviation in the PIPC values, no significant difference between the SHT and control groups was recognized in the statistical analysis (Fig. 4). Except for the fact that the SHT-group showed slightly lower values of the P III P on the 14th postoperative day than the control group, there were no significant differences noted in the values assayed on other days between the two groups (Fig. 5).

(2) Type-IV collagen

The 7S level on the first postoperative day showed no significant difference between the SHT and control

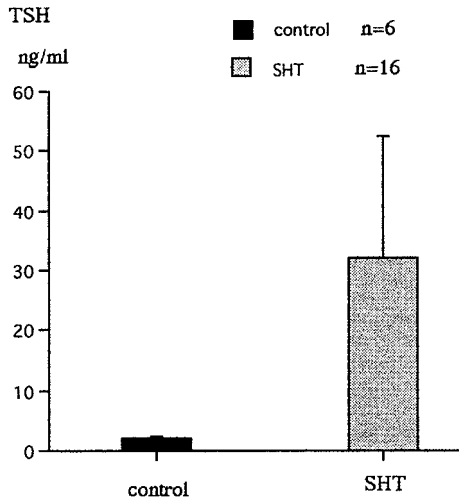


Fig. 3 Mean (±SD) content of TSH in two groups after surgical management, Mean (±SD) content of TSH in control group: 2.667 ± 1.131 , Mean (±SD) content of TSH in SHT group: 33.725 ± 18.378 , P value < 0.001

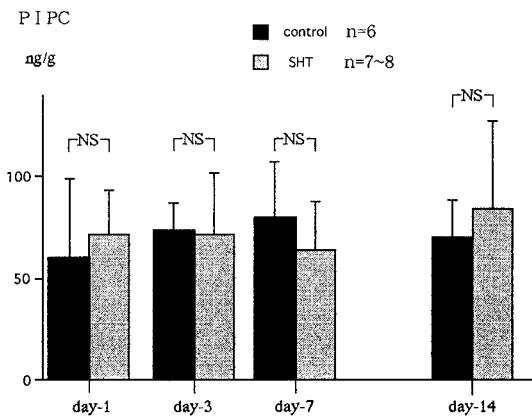


Fig. 4 Mean (±SD) content of P I P C in the two groups after surgical management

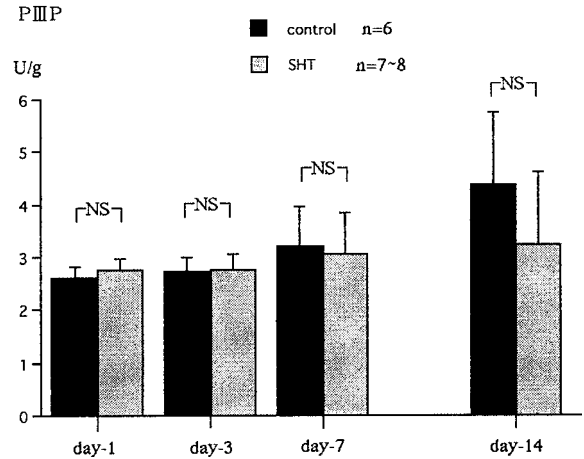


Fig. 5 Mean (±SD) content of P I I P in the two groups after surgical management

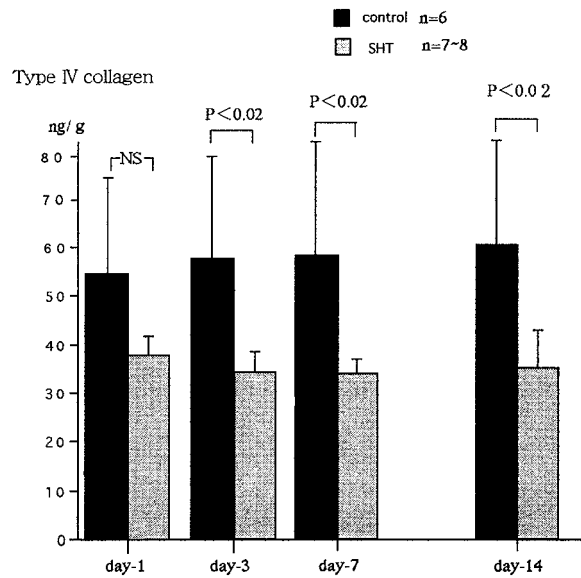


Fig. 6 Mean (±SD) content of type IV collagen in the two groups after surgical operation

groups, but the level was statistically low on the 3rd, 7th, and 14th postoperative days in the SHT-group (Fig. 6).

(3) Hydroxyproline

The hydroxyproline content in the SHT-group was statistically low on the 7th and 14th postoperative days as compared to that of the control group (Fig. 7).

Discussion

Several clinical reports have shown that the delay of wound healing in hypothyroid patients was improved by T 4 administration^{3,8-10} and that there was a correlation between the tensile strength in the wound and thyroid function¹¹. The authors¹² have previously

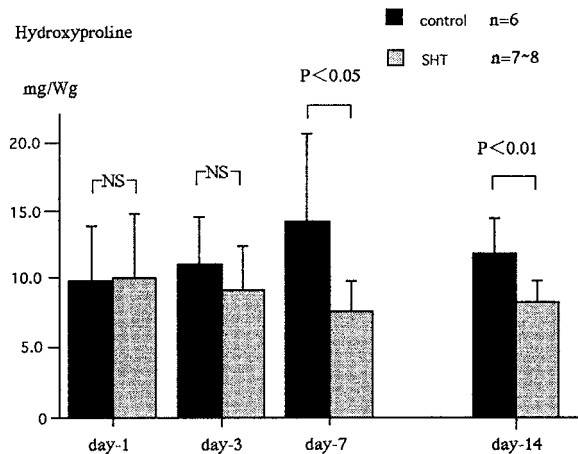


Fig. 7 Mean (±SD) content of hydroxyproline in the two groups after surgical operation

reported the influence of hypothyroidism on wound healing by measuring hydroxyproline, but they were unable to produce satisfactory results. The most probable reason for this was that in the previous experiment a hypothyroid model could not be satisfactorily produced by the mere administration of anti-thyroid drugs. In this current study, however, we believed that a state of hypothyroidism was better and completely developed in the rats by totally resecting the thyroid gland.

The process of wound healing can, in general, be divided into three phases: the inflammatory, proliferative, and remodeling phase. The inflammatory phase takes place from the initial day to the third day following injury. The cells involved during this period are constituted mainly by inflammatory cells, such as granulocytes and monocytes. The subsequent proliferative phase takes place from the third day to the second week after injury, and the main cells involved are the fibroblasts. During the remodeling phase which extends from the second week to several months, fibrocytes cells are the main cells involved. As collagen increases rapidly until about the third week and then levels off thereafter, the wound will by then have gained enough tensile strength. Factors known to interfere with the process of wound healing include interfere of the phagocytotic ability of granulocytes, fibroblast proliferation, synthesis of collagen in diabetes mellitus, prolongation of the inflammatory phase, development of fibrosis in a state of hypoproteinemia, and the production of improper procollagen by the reduction in the hydroxide of proline and ricin in vitamin C deficiency.

In the incipient stage of wound healing, it is known that the mutual binding of coagulation factor-XIII, fibronectin and collagen around fibrin, forms the fibrin matrix. The collagen fiber secreted from the fibroblast during the inflammatory phase and right up to the proliferative phase is the main element that maintains the tensile strength of the wound. In wound healing, tensile strength shows an extremely clear correlation with hydroxyproline.

The authors have paid special attention to the following: hydroxyproline as an index of wound healing in both the inflammatory and proliferative phases; type-I collagen as the main collagen of the skin, bone and tendon; type-III collagen recognition in skin and blood vessels; and type-IV collagen being the main

element of the basement membrane. In our experiment, we thus studied how the state of hypothyroidism influenced wound healing by measuring each type of collagen.

It has been reported¹² that PIPC in type-I collagen is elevated in the process of wound healing until the 7th postoperative day and it shows a time correlation with P III P. In our experimental results, the PIPC gently rose until the 7th postoperative day in the control group, showing a correlation with the P III P change in the control group. In the SHT-group, however, the PIPC slightly decreased until the 7th postoperative day, and reached a peak on the 14th postoperative day indicating no correlation with the P III P.

Regarding type-III collagen, P III P is known to achieve an abnormally high value in hyperthyroidism¹³; however, it remains obscure in hypothyroidism. As described earlier, it has been reported that P III P in general is elevated in wounds. In our experiment, the P III P also showed a gentle rise in the skin tissue obtained between the inflammatory and proliferative phases in both groups. Although the P III P in the SHT-group only showed a lower value than the control group on the 14th postoperative day, no significant difference was recognized between the two groups before the 14th postoperative day.

Senda et al.¹⁴ reported that type-IV collagen is elevated in hyperthyroidism, while the decreased type-IV collagen in hypothyroidism recovers with T 4 administration. They thus speculated that type-IV collagen is influenced by thyroid function. The 7 S content of the skin obtained from the SHT-group indicated a low value on the 1st, 3rd, 7th, and 14th postoperative days compared with that from the control group. A significant decrease was noted especially on the 3rd, 7th and 14th postoperative days. There were anticipated results, as we predicted that 7S would decrease in hypothyroidism.

According to the author's experimental results, hydroxyproline kept rising until the 7th postoperative day in the control group. However, the hydroxyproline content was statistically and significantly low in the SHT-group on the 7th and 14th postoperative days compared with that in the control group. Since hydroxyproline, an index of collagen metabolism, is reported to indicate an extremely direct correlation with the tensile strength in wound healing, we can safely assume that in the SHT-group rats there is a

decrease in tensile strength and delay in wound healing.

According to the results of our experimental study, we conclude the following: 1) A definite state of hypothyroidism was able to be surgically developed (SHT-group). 2) The results of the assay of collagen and hydroxyproline in wounds indicated a significant decrease in type-IV collagen and hydroxyproline in the SHT-group during the inflammatory phase and extending to the proliferative phase.

These findings suggest that thyroid hormone is associated with the proliferation and secretion of fibroblasts in the process of wound healing. In the state of hypothyroidism, it appears that the suppression of thyroid hormone secretion causes a disturbance in the metabolic activation in tissues and the synthesis of collagen extending from the inflammatory phase to the proliferative phase. On the basis of the results obtained from our study, the state of hypothyroidism is a contributing factor to the delay in wound healing.

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