Kinetic Analysis of the Rate of Corneal Wound Healing in Otsuka Long-Evans Tokushima Fatty Rats, a Model of Type 2 Diabetes Mellitus

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Abstract: Diabetic keratopathy is a well-known ocular complication secondary to type 2 diabetes mellitus. In this study, we performed a kinetic analysis of corneal wound healing in Long-Evans rats (normal rat) and Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a model of type 2 diabetes mellitus. Corneal wound healing in 7-week-old normal rats was mostly complete 24 h after corneal epithelial abrasion, and the process of corneal wound healing took place according to an equation with a first-order rate constant. The rate of corneal wound healing in normal rats decreased with aging. The process of corneal wound healing in 38- and 60-week-old normal and OLETF rats occurred in two phases with rate constants for the first and second phases represented as α and β, respectively. The α and β values in 38- and 60-week-old OLETF rats were lower than those in normal rats of the corresponding age. Furthermore, a close relationship was observed between the corneal wound healing rate constant and plasma glucose levels in OLETF rats. The present studies suggest the sequence of events that occur following damage to the corneal surface in OLETF rats as a model animal for a human type 2 diabetes mellitus.

Key words: cornea, corneal wound healing, type 2 diabetes mellitus, glucose, Otsuka Long-Evans Tokushima fatty rat

1 INTRODUCTION

The long-term use of eye drops containing benzalkonium chloride (BAC), which is a surface-active agent, causes corneal epithelium damage, and it is known that the use of eye drops containing BAC strongly relate the corneal wound healing rate in patients with diabetic mellitus¹. However, it was not reported for the in vivo analysis method of corneal wound healing process. Therefore, the development of in vivo analysis method is highly expected to kinetical elucidation of the corneal wound healing mechanism in diabetes mellitus.

The prevalence of type 2 diabetes mellitus is increasing rapidly, and currently affects the health of millions of humans, and will continue to do so in the near future. Among the factors responsible for the increasing prevalence of this disease are obesity, the consumption of energy-dense diets and low levels of physical activity². Type 2 diabetes mellitus results from the failure of pancreatic beta cells to compensate adequately for obesity and insulin resistance³. Type 2 diabetes mellitus leads to a variety of secondary complications with diabetic keratopathy leading to the well-known ocular complications⁴. Diabetic keratopathy is a pathology that includes slow healing or loose adhesion of the corneal epithelium after wounding in diabetic patients. Histologically, it involves a thickening of the corneal epithelial basement membrane and morphologic changes in the corneal epithelium and endothelium⁵⁻¹⁰. Clinically, damage to the corneal epithelium during the use of eye drops treatment, vitreous surgery and retinal photocoagulation sometimes induces vision-threatening corneal complications, such as persistent epithelial defects in diabetic patients¹¹. It has been reported that such diabetic keratopathy is experienced by 50% or more of diabetic patients¹². For analyses of the kinetics of diabetic keratopathy, the selection of the experimental animal is very important. In general, GK rats are used as a model for type 2 diabetic keratopathy¹³. However, GK rats develop type 2 dia-
betic mellitus in the absence of metabolic syndrome. The
Otsuka Long-Evans Tokushima Fatty (OLETF) rat, a model
of type 2 diabetes mellitus, is an established model of hu-
man type 2 diabetes mellitus via metabolic syndrome\(^{14}\).
Nearly 100\% of male OLETF rats develop a diabetic syn-
drome by 25 weeks of age. Hyperglycemia and hyperinsu-
linemia are exhibited in the early phases of the disease as a
result of islet cell hyperplasia and peripheral insulin resist-
tance, and 38-week-old OLETF rats are used as a model of
human type 2 diabetes mellitus\(^{15,18}\). With continued aging,
the rats eventually develop hypoinsulinemia as a result of
the deterioration of islet beta cells\(^{16,18}\), and the plasma
insulin level in OLETF rats over 60 weeks of age is lower
than that in Long-Evans rats of the corresponding age used
as normal controls\(^{19,20}\). The changes in the biological char-
acteristics of OLETF rats show an obvious correspondence
with those that take place in human type 2 diabetes mellitus.

In this study, we performed kinetic analyses of corneal
wound healing in normal and OLETF rats with aging. In ad-
dition, we investigated the relationship between the rate of
corneal wound healing and some blood chemistry values
for diabetes mellitus in OLETF rats with type 2 diabetes mel-
litus.

2 EXPERIMENTAL

2.1 Animals

Male Long-Evans rats (normal rats) or OLETF rats aged
7, 38 and 60 weeks old were used in this study. The normal
and OLETF rats were obtained from Otsuka Pharmaceutical
Co., Ltd., and housed under standard conditions (12 h/d
fluorescent light (07:00-19:00), 25°C room temperature)
with free access to a commercial diet (CE-2, Clea Japan
Inc., Tokyo, Japan) and water. All procedures were per-
formed in accordance with the guidelines of the Kinki Uni-
versity School of Pharmacy Committee for the Care and
Use of Laboratory Animals.

2.2 Blood test for diabetes mellitus

Rats were fasted for 15 h, after which blood was drawn
from a tail vein at AM 9:00 without anesthesia, and plasma
glucose (Glu), triglycerides (TG), total cholesterol (Cho)
and insulin levels were measured. The Glu and TG levels
were determined using an AccuTrend GCT (Roche Diag-
nostics, Mannheim, Germany). Cho levels were measured by
the cholesterol oxidase method and the Phosphotungstate-
magnesium salt method using a Cholesterol E-Test Kit
(Wako, Osaka, Japan). Insulin levels were measured using
an ELISA Insulin Kit according to the manufacturer’s in-
structions (Morinaga Institute of Biological Science Inc.,
Kanagawa, Japan). Briefly, monoclonal antibodies specific
for rat insulin were pre-coated onto microplates, standards
and samples were pipetted into the wells, and the micro-
plates were incubated at 4°C for 2 h. After washing to
remove unbound materials, rat insulin antibodies were added
to the wells at room temperature for 30 min. After wash-
ing, the substrates were added. The enzyme reactions
yielded blue products that turned yellow when the stop solu-
tions were added. The absorbance was measured with a
microplate reader (BIO-RAD, California, USA) at 450 nm.

2.3 Image analysis of corneal wound healing in normal
and OLETF rats

Corneal epithelium debridement of normal and OLETF
rats was performed as described previously\(^{21}\). Normal and
OLETF rats were anesthetized with pentobarbital (30 mg/
kg, i.p.), and a 3.5-mm-diameter circle was outlined in the
center of the cornea with a disposable dermatological skin
knife (BIOPSY PUNCH, Kai industries Co. Ltd, Gifu, Ja-
pain). The encircled corneal epithelium was removed with a
BD Micro-Sharp\(^{22}\) (blade 3.5 mm, 30º, Becton Dickinson,
Fukushima, Japan). The areas of the removed corneal epi-
thelium were as follows: 7-week-old normal rats, 10.72 ±
0.22; 38-week-old normal rats, 9.73 ± 0.19; 38-week-old
OLETF rat, 9.62 ± 0.41; 60-week-old normal rats, 10.05 ±
1.21; 60-week-old OLETF rats, 10.48 ± 0.43 (mm\(^2\); means ±
S.E. of 4 independent rat corneas). The wounds produced
by the removal of corneal epithelium in the 7-, 38- or
60-week-old normal and OLETF rats were washed with a
solution containing 1% fluorescein (Alcon, Tokyo, Japan)
and 0.4% Benoxil (Santen Pharmaceutical Co., Ltd., Osaka,
Japan)\(^{21}\). Changes in the corneal wounds were monitored
using a TRC-50X (Topcon, Tokyo, Japan) equipped with a
digital camera (EOS Kiss Digital N, Canon Inc., Tokyo, Ja-
pain)\(^{21}\), and analyzed with image \(J^{22}\). The remaining corne-
al wound (%) was calculated according to Eq. 1:

\[
\text{Corneal wound (\%)} = \frac{\text{wound area}_{0-72 h}}{\text{wound area}_{0 h}} \times 100
\]  

(1)

The rate of corneal wound healing is represented by the
corneal wound healing rate constant \((k_i, \alpha \text{ and } \beta, \text{ h}\(^{-1}\))\). The
corneal wound healing rate constant was calculated from
the following Eq. 2-1 and 2-2 and the iterative nonlinear
least-squares regression procedure MULTIT\(^2\).

\[
W_i = W_0 \cdot e^{-k_i \cdot t}
\]

\[
W_i = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t}
\]

(2-1)

(2-2)

where \(t\) is time (0-72 h) after corneal abrasion, and \(W_i\) is the
percentage of corneal wound (\%) at the corresponding
time. \(W_0\) is the percentage of corneal wound (\%) at 0.
\(\alpha\) and \(\beta\) show the corneal wound healing rate constants
in the first and second-phases, respectively. \(A\) and \(B\) are the
corneal wound areas (\%) in the \(\alpha\)-and \(\beta\)-phases, respec-
tively. \(A\) and \(B\) indicate the contribution ratio of the corneal
wound healing processes for the \(\alpha\)-and \(\beta\)-phase, respec-
tively.
2.4 Statistical analysis

All values are represented as mean ± standard error of the mean (S.E.). Unpaired Student’s or Aspin-Welch’s t-tests were used to evaluate statistical differences, and multiple groups were evaluated by one-way analysis of variance followed by Dunnett’s multiple comparison. P values less than 0.05 were considered significant.

3 RESULTS

3.1 Rates of corneal wound healing in normal and OLETF rats with aging

Figures 1A and 1B shows corneal wound levels after corneal epithelial abrasion in 7-, 38- and 60-week-old normal or OLETF rat corneas. The corneal wounds of 7-week-old normal rat were approximately 30% healed 12 h after abrasion, and were almost entirely healed 24 h after corneal epithelial abrasion. The corneal wounds of 38- and 60-week-old normal rats were almost entirely healed 48 h after corneal epithelial abrasion. The corneal wound healing rate in 38- and 60-week-old OLETF rats was slower than in normal rats of the corresponding age, and the corneal wounds were healed by 72 h after corneal epithelial abrasion. Table 1 shows the Akaike’s information criterion (AIC) for kinetic analysis using Eq. 2-1 and 2-2 for corneal wound healing in 7-, 38- and 60-week-old normal or OLETF rats after corneal epithelial abrasion; Table 2 shows the rate constants and contribution ratios for corneal wound healing in 7-, 38- and 60-week-old normal or OLETF rats after corneal epithelial abrasion. From the AIC results, the values ($k_w$) of the corneal wound healing rate constant over the period 0-72 h (0-36 h for 7-week-old normal rats) after corneal epithelial abrasion were calculated using Eq. 2-1, and the values ($\alpha$ and $\beta$) of the corneal wound healing rate constants over the period 0-72 h after corneal epithelial abrasion for 38- and 60-week-old normal and OLETF rats were calculated using Eq. 2-2. The values of the corneal wound healing rate constants ($k_w$, $\alpha$ and $\beta$) for 38- and 60-week-old normal rats were similar as analyzed by Eq. 2-1 and 2-2. Therefore, the same analytical method used for OLETF rats (Eq. 2-2) was also applied to the analyses of 38- and 60-week-old normal rats for comparison of the corneal wound healing rate process in OLETF rats in this study. The corneal wound healing rate constants for 38-week-old normal rats were similar to those for 60-week-old normal rats.

<table>
<thead>
<tr>
<th>Rat</th>
<th>Age (week)</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eq. 2-1</td>
<td>Eq. 2-2</td>
</tr>
<tr>
<td>Normal</td>
<td>7</td>
<td>44.7 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>45.0 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>46.4 ± 1.5</td>
</tr>
<tr>
<td>OLETF</td>
<td>38</td>
<td>43.7 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>48.1 ± 0.5</td>
</tr>
</tbody>
</table>

The calculation of values for 7-week-old normal rats is based on the period 0-36 h after corneal epithelial abrasion; the values for 38- and 60-week-old rats are based on the period 0-72 h after corneal epithelial abrasion. The data are presented as means ± S.E. of 4 independent rats.

Table 2 Kinetic Analysis of the Rate of Corneal Wound Healing in 38- and 60-Week-Old Normal and OLETF Rats After Corneal Epithelial Abrasion Using Eq. 2-1 and 2-2

<table>
<thead>
<tr>
<th>Eq. 2-1</th>
<th>Normal (7 weeks)</th>
<th>Normal (38 weeks)</th>
<th>OLETF</th>
<th>Normal (38 weeks)</th>
<th>OLETF</th>
</tr>
</thead>
<tbody>
<tr>
<td>$W_0$ (%)</td>
<td>110.9 ± 2.0</td>
<td>105.3 ± 1.7</td>
<td>102.4 ± 0.7</td>
<td>106.2 ± 0.9</td>
<td>105.2 ± 1.6</td>
</tr>
<tr>
<td>$k_w$ ($\times 10^{-3}$, h$^{-1}$)</td>
<td>71.0 ± 4.5</td>
<td>48.5 ± 3.5$^{11}$</td>
<td>33.9 ± 1.2$^{12}$</td>
<td>46.3 ± 2.5$^{11}$</td>
<td>36.9 ± 4.0$^{13}$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Eq. 2-2</th>
<th>Normal (7 weeks)</th>
<th>Normal (38 weeks)</th>
<th>OLETF</th>
<th>Normal (38 weeks)</th>
<th>OLETF</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A$ (%)</td>
<td>111.0 ± 2.4</td>
<td>81.2 ± 10.6$^{14}$</td>
<td>67.4 ± 8.9</td>
<td>78.6 ± 8.9$^{13}$</td>
<td>65.8 ± 15.7</td>
</tr>
<tr>
<td>$\alpha$ ($\times 10^{-3}$, h$^{-1}$)</td>
<td>71.1 ± 5.2</td>
<td>48.0 ± 4.9$^{17}$</td>
<td>35.2 ± 2.2$^{11}$</td>
<td>47.1 ± 2.7$^{11}$</td>
<td>38.2 ± 3.2$^{11}$</td>
</tr>
<tr>
<td>$B$ (%)</td>
<td>4.9 ± 2.7</td>
<td>24.2 ± 9.4</td>
<td>35.0 ± 8.4</td>
<td>26.8 ± 8.9</td>
<td>38.2 ± 15.2</td>
</tr>
<tr>
<td>$\beta$ ($\times 10^{-3}$, h$^{-1}$)</td>
<td>17.8 ± 14.2</td>
<td>47.6 ± 3.5</td>
<td>32.2 ± 1.2$^{12}$</td>
<td>47.0 ± 2.7</td>
<td>35.8 ± 2.5$^{13}$</td>
</tr>
</tbody>
</table>

The rate constants of corneal wound healing ($k_w$, $\alpha$ and $\beta$) and contribution ratios ($W_0$, $A$ and $B$) for the period 0-72 h after corneal epithelial abrasion were calculated using Eq. 2-1 and 2-2. The data are presented as means ± S.E. of 4 independent rats.$^{14}$ P < 0.05 vs. 7-week-old normal rats for each category.$^{15}$ P < 0.05 vs. 38-week-old normal rats for each category. $^{14}$ P < 0.05 vs. 38-week-old normal rats for each category.$^{16}$ P < 0.05 vs. 60-week-old normal rats for each category. $^{14}$ P < 0.05 vs. B of 38-week-old normal rats.$^{17}$ P < 0.05 vs. B of 60-week-old normal rats.

old normal rats. However, the corneal wound healing rate constants were lower than for 7-week-old normal rats. The $\alpha$ and $\beta$ values for 38-and 60-week-old OLETF rats were smaller than those for normal rats at the corresponding ages. In addition, the $\beta$ values for the OLETF rat were significantly lower in comparison with normal rats.

Fig. 1 Corneal Wound Healing in 7-, 38- and 60-Week-Old Normal and OLETF Rats.

The corneal epithelium was removed with a BD Micro-Sharp™, and the resulting corneal wounds were dyed with 1% fluorescein solution. (A) Photographs of 7-, 38- and 60-week-old normal and OLETF rat eyes stained with fluorescein. (B) Corneal wound (%) of 7-, 38- and 60-week-old normal and OLETF rat corneas. Open circles, 7-week-old normal rats; open triangles, 38-week-old normal rats; closed triangles, 38-week-old OLETF rats; open squares, 60-week-old normal rats; closed squares, 60-week-old OLETF rats. The data are presented as means ± S.E. of 4 independent rats. *1 $P < 0.05$ vs. 7-week-old normal rats. *2 $P < 0.05$ vs. 38-week-old normal rats. *3 $P < 0.05$ vs. 60-week-old normal rats.
Table 3 Body Weight and Some Blood Test Values for Diabetes Mellitus in 38- and 60-Week-Old Normal and OLETF Rats

<table>
<thead>
<tr>
<th></th>
<th>38-week-old</th>
<th></th>
<th>60-week-old</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>OLETF</td>
<td>Normal</td>
<td>OLETF</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>488.6 ± 14.2</td>
<td>621.3 ± 19.7</td>
<td>526.3 ± 33.0</td>
<td>416.3 ± 17.4</td>
</tr>
<tr>
<td>Glu (mg/dl)</td>
<td>119.3 ± 4.9</td>
<td>213.5 ± 15.7</td>
<td>140.8 ± 3.6</td>
<td>244.3 ± 23.9</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>128.0 ± 9.3</td>
<td>419.8 ± 22.2</td>
<td>150.0 ± 14.4</td>
<td>335.8 ± 11.0</td>
</tr>
<tr>
<td>Cho (mg/dl)</td>
<td>101.4 ± 11.4</td>
<td>209.2 ± 11.1</td>
<td>83.6 ± 14.3</td>
<td>274.5 ± 26.2</td>
</tr>
<tr>
<td>Insulin (ng/dl)</td>
<td>105.5 ± 11.6</td>
<td>237.4 ± 26.6</td>
<td>111.1 ± 6.7</td>
<td>83.0 ± 7.2</td>
</tr>
</tbody>
</table>

The data for 38-week-old normal and OLETF rats have been reported in ref. 43; the data represent remeasurements made to compare blood test values for diabetes mellitus in 38- and 60-week-old normal and OLETF rats. The data are presented as means ± S.E. of 4 independent rats.*P < 0.05 vs. 38-week-old normal rats for each category. **P < 0.05 vs. 60-week-old normal rats for each category.

Table 4 Correlation Coefficients (r) among the Corneal Wound Healing Rate Constants (α and β) and Body Weight, Glu, TG, Cho and Insulin Levels in 38- and 60-Week-Old OLETF Rats

<table>
<thead>
<tr>
<th>Relationship</th>
<th>α</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>vs. body weight</td>
<td>-0.46</td>
<td>-0.40</td>
</tr>
<tr>
<td>vs. Glu</td>
<td>-0.71*</td>
<td>-0.72*</td>
</tr>
<tr>
<td>vs. TG</td>
<td>-0.31</td>
<td>-0.42</td>
</tr>
<tr>
<td>vs. Cho</td>
<td>0.47</td>
<td>0.43</td>
</tr>
<tr>
<td>vs. insulin</td>
<td>-0.29</td>
<td>-0.27</td>
</tr>
</tbody>
</table>

The α and β values for the period 0-72 h after corneal epithelial abrasion were calculated using Eq. 2-2. The data represent values for 8 independent rats. *P < 0.05.

Fig. 2 Relationship between the Corneal Wound Healing Rate Constants (α and β) and Glu Levels in 38- and 60-Week-Old OLETF Rats.

Corneal wound healing rate constants (α and β) 0-72 h after corneal epithelial abrasion were calculated using Eq. 2-2. Glu was determined by the Accutrend GCT (Roche Diagnostics). (A) Relationship between α values and Glu levels. (B) Relationship between β values and Glu levels. Open circles, 38-week-old OLETF rats; closed circles, 60-week-old OLETF rats.
4 DISCUSSION

In this study, we performed a kinetic analysis of corneal wound healing in normal and OLETF rats with aging. In addition, we investigated the relationship between the corneal wound healing rate and some blood test values for diabetes mellitus in OLETF rats with type 2 diabetes mellitus.

The maintenance of corneal epithelial cell mass can be viewed as the result of three separate, independent phenomena. Thoft and Friend have termed these: X, the proliferation of basal epithelial cells; Y, the contribution to the cell mass of the centripetal movement of peripheral cells; and Z, epithelial cell loss from the surface. Corneal epithelial maintenance thus can be defined by the equation: \( X + Y = Z \), which simply states that if the corneal epithelium is to be maintained, cell loss must be balanced by cell replacement. The corneal wound healing process is divided into three sequential and partially overlapping steps: epithelial cell loss from the surface (Z) reduces and eventually covers the wound surface (Y), while cell proliferation (X) provides cells to rebuild the tissue and tissue remodeling to restore the stratified epithelium. In this study, corneal wound healing in 7-week-old normal rats was completed by 24 h after corneal epithelial abrasion. Corneal wound healing in normal rats became slower with aging; however, the corneal wound healing rates of 38-week-old normal rats were similar to those of 60-week-old normal rats, suggesting that the decrease in the rate of corneal wound healing with aging may reach a plateau. The corneal wounds of OLETF rats were repaired more slowly following corneal epithelial abrasion than in normal rats. These results suggest that the development of type 2 diabetic mellitus is associated with a decrease in the rate of corneal wound healing.

Corneal wound healing in 38- and 60-week-old OLETF rats takes place in clear two phases. It has been reported that the early stages of epithelial wound closure rely predominantly on cell migration rather than cell proliferation; cell proliferation starts approximately 24 h after corneal epithelial injury, after which tissue remodeling to restore the stratified epithelium occurs. Furthermore, Zagon et al. showed that the corneal wounds of Sprague-Dawley rats (250–300 g) were covered due to cell migration by 24 h corneal epithelial injury. After that, the interior damage was healed by cell proliferation. Consistent with the results of Zagon et al., the corneal wounds of 7-week-old normal rat (248 ± 4.17, g, mean ± S.E. of 4 independent rats) were also nearly healed 24 h after corneal epithelial abrasion in this study. On the other hand, corneal wound healing in normal rats was delayed with aging, and the wound surfaces in 38- and 60-week-old normal and OLETF rats were not completely covered by cell migration 24 h corneal epithelial injury. The second-phase of healing, involving cell movement and proliferation, may be the source of the delay in corneal wound healing in the 38- and 60-week-old OLETF rats. In addition, aging may result in a decrease in cell migration, since the corneal wound healing rate constant of 38- and 60-week-old normal rats was lower than that of 7-week-old normal rats.

In this study, we analyzed the two phases of corneal wound healing in 38- and 60-week-old normal and OLETF rats.

![Scheme 1](image_url)

**Scheme 1** The Function of Cell Migration and Proliferation in Corneal Wound Healing in 7-, 38- and 60-Week-Old Normal and OLETF Rats.

The movement of superficial cells shows cell migration, and the number of basal cells represent cell proliferation.
rats using Eq. 2-2, and calculated the corneal wound healing rate constants of the first and second-phases (α and β). The β values for 38-and 60-week-old OLETF rats were significantly lower than those for normal rats at corresponding ages. The contribution ratio A of the corneal wound healing process to the α-phase in 38-and 60-week-old normal rats was significantly higher than the ratio B of the corneal wound healing process for the second-phase. On the other hand, the contribution ratio B of the corneal wound healing process for the β-phase in 38-and 60-week-old OLETF rats tended to increase. We hypothesize that α represents cell movement, which is the main wound healing process up to 24 h after corneal epithelial abrasion, while the β-phase, which takes place 18 – 72 h after corneal epithelial abrasion, represents cell proliferation. A deficit in cell proliferation may be predominantly responsible for the delay in corneal wound healing in this model. On the other hand, the α and β values for 38-and 60-week-old normal rats were similar, and the contribution to the corneal wound healing process of ratio A for the α-phase is significantly higher than the B for the β-phase. This result suggests that the corneal wounds of old normal rats are repaired by both cell movement and proliferation, and the rate of healing wound becomes equivalent once cell proliferation begins (Scheme 1).

The body weights of 38-week-old OLETF rats were approximately 1.3-fold those of 38-week-old normal rats. Glu, TG and Cho levels in 38-week-old OLETF rats increased with age, and their levels were all significantly higher than in 38-week-old normal rats; plasma insulin levels in 38-week-old OLETF rats were also higher than in 38-week-old normal rats (Table 3). These results indicate that the 38-week-old OLETF rats used in this study developed diabetes mellitus via insulin resistance. Consistent with the results of 38-week-old OLETF rats, the body weight and the plasma insulin levels of the 60-week-old OLETF rats were significantly lower than those of the 60-week-old normal rats. This suggests that the type 2 diabetes mellitus in 60-week-old OLETF rats has reached a fairly advanced stage, and the changes may be caused to the deterioration of islet beta cells with the progression of the type 2 diabetes mellitus. On the other hand, the β values for 38-and 60-week-old OLETF were similar. Therefore, it was determined that the progression of type 2 diabetes mellitus does not affect the process of the corneal wound healing in OLETF rat.

It is important to understand the mechanisms underlying the delay in corneal wound healing in type 2 diabetic mellitus. In diabetes, the levels of glucose in the cornea and tears are increased. Glucose levels in the corneal epithelium have been reported to be 6-fold higher (1.8 to 12.2 μmol/g dry wet.) in diabetic patients than in normal controls[36], and large increases in the glucose content of tears (range 2.16 – 9.55 mg/dL and 14.69 – 27.02 mg/dL for normal and diabetic patients, respectively) have also been reported[19-20]. March et al. reported that the glucose content of tears is approximately 10% the plasma glucose level, and that the glucose content of tears follows changes in plasma glucose levels[40]. High glucose levels suppress the cellular behavior (cell movement and proliferation) of human corneal epithelial cells[41]. In addition, it has been reported that the instillation of insulin normalizes the delay in corneal wound healing in streptozotocin rats[12]. These previous reports indicate that the decrease in corneal wound healing in diabetic keratopathy is caused by a suppression of cell movement and proliferation due to high glucose levels in tears. In this study, a close relationship was observed between the α, β values and Glu levels in 38-and 60-week-old OLETF rats, unlike the progression of type 2 diabetic mellitus (Table 4). This result supports the previous findings for human diabetic keratopathy[42].

Further studies are needed to clarify the effects of BAC on corneal wound healing in diabetic keratopathy, since the use of eye drops containing BAC strongly relate the corneal wound healing rate in diabetic patient. Therefore, we are now investigating the effects of BAC on corneal wound healing in diabetic ulcers using the OLETF and this kinetic analysis.

5 CONCLUSION
The present study predicts the sequence of events that occur following damage to the corneal surface in OLETF rats as a model animal for human type 2 diabetes mellitus using the fluorescein staining method and kinetic analysis of the rate of corneal wound healing. First, the corneal wound healing rate in young normal rats (7-week-old rat) is very rapid, and can be analyzed as a first-order equation with one phase. This means that wound closure by epithelial cell migration is complete within 24 h after corneal epithelial abrasion. Second, corneal wound healing in older normal rats (38-and 60-week-old rats) occurs in two phases (α and β) showing little difference, with cell migration until 24 h after corneal epithelial abrasion, and then cell replication occurring simultaneously with centripetal migration during re-epithelialization, an observation originally made in tissue culture studies by Chan et al.[42]. Third, the wound healing rate in 38-and 60-week-old OLETF rats occurs in two obvious phases, both slower than those in old normal rats of the corresponding age. Fourth, a close relationship is observed between the corneal wound healing rate constants and Glu levels in OLETF rats. These findings provide information significant for designing further studies to develop potent drugs to improve the corneal wound-healing ability of diabetic patients.
References


Kinetic Analysis of the Rate of Corneal Wound Healing


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