Expression and activity levels of chymase in mast cells of burn wound tissues increase during the healing process in a hamster model

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Abstract. The present study aimed to investigate the changes in the expression levels and activity of mast cell chymase in the process of burn wound healing in a hamster model of deep second-degree burn. The hamster model was established by exposing a ~3 cm diameter area of bare skin to hot water (75˚C) for 0, 6, 8, 10 or 12 sec. Tissue specimens were collected 24 h after burning and histological analysis revealed that hot water contact for 12 sec was required to produce a deep second-degree burn. Quantitative polymerase chain reaction and a radioimmunoassay were used to determine changes in chymase mRNA expression levels and activity. The mRNA expression levels and activity of chymase were increased in the burn wound tissues when compared with the normal skin. However, no statistically significant differences were observed in mast cell chymase activity amongst the various post-burn stages. Chymase mRNA expression levels peaked at day 1 post-burn, subsequently decreasing at days 3 and 7 post-burn and finally increasing again at day 14 post-burn. In summary, a hamster model of deep second-degree burn can be created by bringing the skin into contact with water at 75˚C for 12 sec. Furthermore, the mRNA expression levels and activity of chymase in the burn wound tissues increased when compared with those in normal skin tissues.

Introduction

Skin wound healing is a complex multifactorial process, involving inflammation, cell proliferation, cell migration, re-epithelialization, angiogenesis, extracellular matrix deposition and remodeling (1,2). Burn wounds have a long healing period, and are the most representative persistent wounds for use in the study of skin wound healing. Deep second-degree burn wounds exhibit spontaneous epithelial regeneration through the proliferation and migration of the skin appendages under the wound and the epidermal cells around the wound margin (3). The healing period is relatively long; thus, deep second-degree burns are useful for the experimental study of burn wounds. Animal models of burn wounds have been used to study various pathological changes and healing mechanisms involved in the burn wound healing process (4).

Burn-induced tissue ischemia and inflammation can increase the number of mast cells in burn tissues (5) and stimulate mast cells to release vasoactive substances by degranulation (6). Mast cells play a role in the acute inflammatory phase and participate in wound healing together with fibroblasts (7,8). Once activated, mast cells degranulate and release histamine, heparin and a variety of enzymes, such as chymase, cathepsin G and hydroxy peptidase A (9). Chymase is closely associated with tissue fibrosis.

Chymase is an α-chymase-like serine protease that is involved in numerous physiological and pathophysiological processes. One of the most important functions of chymase is the regulation of angiotensin (Ang)II generation. In addition, chymase promotes myocardial and skin fibroblast proliferation (10,11), the release of transforming growth factor-β1 bound to the extracellular matrix and the degradation of procollagen protein that participate in tissue remodeling (12) and inflammation (13). Previous studies have shown that mast cell chymase is active during the healing process in burn wounds (14,15). However, the changes in chymase expression levels and activity in the tissues during burn wound healing remain unclear. Human chymase differs to the chymase in the majority of other animals with regard to substrate specificity, with the exceptions of monkeys, dogs and hamsters (16-18).

To the best of our knowledge, there have been no previous
studies reporting the use of a hamster model in the study of burn wounds. In the present study, a hamster model of deep second-degree burn wound was established in order to study the association between mast cell chymase and the burn wound healing process.

Materials and methods

Hamster model for deep second-degree burn wound. A total of 24 hamsters (weight, 40-60 g; age, 8 weeks) were used, obtained from the Ürümqi Municipal Center for Disease Control and Prevention (Ürümqi, China). All animals were individually housed in stainless steel cages with a 12-h light-dark cycle and a controlled temperature. The hamsters received food and water ad libitum and were acclimatized for at least two weeks prior to thermal injury. All animal care and experimental procedures were approved by the Animal Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Ürümqi, China).

The body surface areas of the hamsters (equivalent to 0.0913 x weight\(^3\)) ranged between 106.8 and 139.9 cm\(^2\) (19). The burn wound was circular, with a diameter of ~3 cm, accounting for 5-6.5% of their body surface area. The hamsters were anesthetized with ketamine (0.7 g/kg), and diazepam and atropine were used to maintain adequate anesthesia. Once anesthetized, the hair on the back of each hamster was removed to reveal a bare region with a diameter of ~3 cm. There are a variety of methods for producing deep second-degree burns in animal models (20-24); however, the majority of these methods are adapted to large animals and may be complex. The burn wound depth may be affected by the contact temperature, heat duration, burn area and pressure. Since hamsters are small in size, there is a risk of accidental mortality if the burn is not inflicted correctly. The end of a 50-ml syringe, with no plunger, was used to administer the burn. While the hamster was anesthetized, the end of the syringe was gently pressed onto the bare region on the back of the hamster to keep the hot water inside the syringe (Fig. 1A). Next, 20 ml water (75°C) was poured into the syringe, producing a circular burn wound with a diameter of ~3 cm. The durations of water contact with the back skin of the hamsters were 0, 6, 8, 10 and 12 sec, for the five groups of four hamsters. The wounds of all the hamsters were washed with running water for 1 min and subsequently centrifuged at 30,000 x g for 20 sec at 4°C and the supernatants were discarded. The sediments were then washed with water, air-dried, soaked with hematoxylin for 15 min and washed with water a further three times. Subsequently, the sections were stained with eosin for 3 min and soaked with 95% ethanol I for 3 min, 95% ethanol II for 3 min, 100% ethanol I for 3 min and 100% ethanol II for 3 min. Once the ethanol had dried, the sections were soaked in xylene for 2 h and subsequently mounted using Permount™ Mounting Medium purchased from Multi Sciences (Lianke) Biotech Co., Ltd. (Hangzhou, China).

Quantitative polymerase chain reaction (qPCR). Total RNA was extracted with TRIzol® (Invitrogen Life Technologies, Carlsbad, CA, USA), chloroform, isopropanol and 75% ethylene glycol, and stored in a refrigerator at -80°C. Reverse transcription was conducted by adding 1.5 µg sample RNA to a 20-µl reaction system that included 1 µl Oligo (dT) Primer, 1 µl reverse transcriptase and 4 µl buffer (Takara Biotec Co., Ltd., Dalian, China). The reaction mixture was incubated for 1 h at 37°C and then heated to 85°C for 5 min to terminate the reaction. For qPCR amplification, 5 µl reaction solution was extracted and added to a 20-µl reaction system that included 10 µl SYBR Premix Ex Taq, 0.25 µl target primer/0.25 µl β-actin primer (Takara Biotec Co., Ltd.) and 4.5 µl ddH\(_2\)O. The reaction was conducted on a Thermal Cycler Dice Real Time System (Takara Bio, Inc., Otsu, Japan).

For the amplification of chymase, the following PCR procedure was used: 95°C for 3 min, 35 cycles of 95°C for 45 sec, 55°C for 45 sec and 72°C for 45 sec, and a final extension at 72°C for 5 min. For the amplification of β-actin, the following PCR procedure was used: 95°C for 4 min, 35 cycles of 95°C for 30 sec, 60°C for 30 sec and 72°C for 2 min, and a final extension at 72°C for 7 min. The PCR product was examined by dissociation curve analysis, and the relative quantification of gene expression was normalized against the internal standard, β-actin.

The primers were designed as follows: Chymase forward, 5'-CTG AGA GGA TGC TTC TTC CTG C-3'; and reverse, 5'-AGA TCT TAT TGA TCC AGG GCC G-3'; β-actin forward, 5'-AAC TCC ATC ATG AAG TGT GA-3'; and reverse, 5'-ACT CCT GCT TGC TGA TCC AC-3'.

Radioimmunoassay. Chymase activity in the burn tissue was determined using a radioimmunoassay (25). Burn tissue samples weighing 100 mg were repeatedly homogenized and added to 50 mM NaH\(_2\)PO\(_4\) buffer (10 w/v, pH 7.4) for 15 min at 4°C. The samples were centrifuged at 3,000 x g for 20 sec at 4°C and the supernatants were discarded. The sediments were added to 50 mM NaH\(_2\)PO\(_4\) buffer for ultrasonic homogenization (400 A, 5 sec/time with an interval of 10 sec, repeated three to five times) and the homogenized tissues were subsequently centrifuged at 30,000 x g for 20 min at 4°C. The homogenization and centrifugation steps were repeated three times and the supernatants, which contained chymase, were collected for each repeat. From each processed sample, 1,500 µl supernatant was collected and divided into three tubes (500 µl each) labeled A, B and C. For reaction system A,
6 ng AngI was added, while 6 ng AngI and 50 µM lisinopril were added to reaction system B and 6 ng AngI and 20 µM aprotinin were added to reaction system C. The tubes were placed in a water bath (37°C) for 15 min, and a 2.5-fold volume of precooled ethanol was added to terminate the reaction. The resulting concentrations of AngII in reaction systems A, B and C were determined using a radioimmunoassay kit (Beijing North Institute of Biological Technology, Beijing, China), according to the manufacturer’s instructions. The activity of angiotensin-converting enzyme (ACE) was calculated by subtracting the enzyme activity of reaction system B from that of A. The activity of other serine proteases was calculated by subtracting the enzyme activity of reaction system C from that of A. Finally, the activity of chymase was calculated by subtracting the ACE activity and the other serine protease activity from the enzyme activity of reaction system A. The enzyme activity required for the generation of 1 nmol AngII in 100 mg burn tissues per min was defined as 1 enzyme activity unit (U). The activity of chymase was expressed as U/mg.

**Statistical analysis.** SPSS software, version 16.0 (SPSS, Inc., Madison, WI, USA) was used for statistical analysis. The results are presented as the mean ± standard deviation. Analysis of variance and Dunnett’s test were used to assess the differences between groups. P<0.05 was considered to indicate a statistically significant difference.

**Results**

A hamster model of deep second-degree burn is established by hot water contact for 12 sec. To evaluate the degree of the burn wounds, hamsters were used as the animal model and H&E staining of the tissues was performed. All hamsters used in the study survived. The degree of paleness of the burnt skin varied...
at different time periods after the injury was inflicted. At 24 h
after the burn was inflicted, the edge of the burn wound was
red with swelling, while the wound itself exhibited swelling
and was white in color, with no visible blisters (Fig. 1B). The
burn contact durations analyzed were 0, 6, 8, 10 and 12 sec.
Histological examination revealed that the epidermal cells
were partially detached from the burn area in the 12-sec burn
duration hamsters. H&E staining showed that the structure of
the tissues was unclear and that blisters had formed on the skin.
In addition, congestion had occurred in the dermal interstitial
layer, collagen fibers had integrated into sheets and residual
hair follicles were observed in the deep dermis. Subcutaneous
vascular dilation, congestion and edema were evident; howev-
er, the tissue structure was not destroyed (Fig. 1C). Thus,
it was determined that the damage caused by 12 sec hot water
contact was equivalent to a deep second-degree burn. Deep
second-degree burns were produced on the four hamsters that
received hot water contact for 12 sec. There were no signs of
infection during wound healing and re-epithelialization was
complete after 18 days (Fig. 1D). These results indicated that a
hamster model of deep second-degree burn was established by
applying hot water contact for 12 sec.

Chymase activity and mRNA expression levels increase in
mast cells after burning and may be involved in the process
of burn wound healing. A radioimmunoassay and qPCR were
used to investigate the activity and mRNA expression levels of
chymase in the burn tissues. Chymase activity was observed
in all the post-burn stages and was significantly higher in the
burnt tissue when compared with the normal skin tissue
(day 0). However, there were no statistically significant differ-
ences in chymase activity amongst the various post-burn
stages (P>0.05; Fig. 2A). At days 1, 3, 7 and 14 after burning,
chymase mRNA expression levels were significantly higher
when compared with those in the normal skin tissue (day 0;
P<0.05). In addition, no statistically significant differences
were observed amongst the chymase mRNA expression levels
of the post-burn stages (P>0.05; Fig. 2B). These results dem-
onstrated that chymase activity and mRNA expression levels
were increased in the mast cells of the burned tissue and may
be involved in the process of burn wound healing.

Discussion

Hamsters were selected as experimental animals to study
chymase activity, as the AngII generated by chymase differs
between species, but is similar in humans and hamsters. The
burns may easily have been fatal to the hamsters; however,
the temperature of the water was controlled and the burn
area selected was easy to manipulate. Thus, hot water at 75°C
was used to produce deep second-degree burn wounds on the
hamsters.

Kim et al (26) used continuous scan laser Doppler vibrometry
to observe the microvascular perfusion conditions of
large-area deep burn wounds in mice and determined the
degrees of the burn wounds according to the recovery of
the mice. Second-degree burns developed into third-degree
burns if the fluid resuscitation rate was ≤4 ml/kg/%burn.
The optimal fluid resuscitation rate was 4-8 ml/kg/%burn and
the perfusion peak was 6 ml/kg/%burn. In the present

study, the burn wounds were washed with water for 5 min to
reduce the residual heat. In addition, 1.5-1.8 ml normal saline
(6 ml/kg/%burn) was administered via intraperitoneal injec-
tion for fluid resuscitation in order to reduce the exacerbation
of the depth of the wounds.

In the present study, the burn area was 7.1 cm², accounting
for 5-6.5% of the body surface area, which was large enough
for the requirements of the study. All hamsters used in the
study survived. In addition, no wound infection or other
complications were found during the healing process of the
deep second-degree wounds. The wounds produced by contact
with 20 ml hot water (75°C) in a 50-ml syringe on the skin of the
hamsters for 12 sec satisfied the requirements of a deep
second-degree burn wound.

Chen et al (27) used qPCR to detect the mRNA expression
levels of cardiac chymase in a hamster model of heart failure,
while Guo et al (28) did the same in ovalbumin-stimulated
atherosclerosis hamsters. Matsumoto et al (29) used qPCR
to detect the mRNA expression levels of chymase in dogs
with tachycardia-induced heart failure following the admin-
istration of the chymase inhibitor, SUNC8257. Notably,
the present study observed increased chymase activity and
mRNA expression levels in the hamsters during the process
of burn wound healing when compared with the levels prior
to the burns. Chymase activity and mRNA expression levels
during the healing process were significantly increased when
compared with the levels prior to the burn model establishment
(P<0.05), while those in different post-burn stages showed no
statistically significant differences (P>0.05). The increased
chymase activity in the burn tissue may have been caused
by a burn-induced stress response in the hamsters, in which
the burns directly acted on the mast cells, activating them to
increase degranulation and release chymase. In addition, the
burns caused increased vascular permeability, and inflamma-
tory responses increased the numbers and sustained activation
of mast cells in the burn wounds.

In conclusion, the results of the present study indicate
that chymase is involved in the process of burn wound healing.
These results may provide experimental evidence for elucidating
the process of burn wound healing and aid the
development of new treatment methods for burn wounds.

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