trans-4-(Aminomethyl)cyclohexane Carboxylic Acid (T-AMCHA), an Anti-Fibrinolytic Agent, Accelerates Barrier Recovery and Prevents the Epidermal Hyperplasia Induced by Epidermal Injury in Hairless Mice and Humans

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Because wounding the epidermis increases proteolytic activity and because disorders associated with barrier dysfunction have elevated protease activity, we studied the effect of protease inhibitors on the time course of barrier recovery and on the development of epidermal hyperplasia induced by repeated injury. After injuries to the epidermis produced by tape stripping, acetone treatment, or detergent (SDS) treatment that disrupt the barrier, a single application of 5% tranexamic acid [4-(aminomethyl)cyclohexane carboxylic acid, t-AMCHA], a well known anti-plasmin reagent, accelerated barrier recovery in both hairless mouse and human skin. In contrast, neither aminocaproic acid nor aminobutyric acid, inactive analogs of t-AMCHA, affected the time course of barrier recovery. Several trypsin-like serine protease inhibitors, e.g., leupeptin, TLCK, and PMSF, also accelerated barrier repair. In contrast other types of protease inhibitors, e.g., EDTA, pepstatin, *N*-ethylmaleimide, chymostatin, and TPCK, did not accelerate barrier recovery. We next evaluated the effects of daily topical application of t-AMCHA on epidermal hyperplasia, induced by repeated tape stripping or acetone treatment for 7 d. The degree of hyperplasia, quantified by the measurement of epidermal thickness, was reduced in both models by repeated applications of t-AMCHA. Finally, proteolytic activity in both human and mouse epidermis increased 1–2 h after epidermal injuries that disrupt the barrier. These results demonstrate that the inhibition of plasmin, a serine protease, accelerates barrier recovery and inhibits the epidermal hyperplasia induced by repeated barrier disruption, perhaps by decreasing the extent of attendant epidermal injury. ***Key words: stratum corneum/transepidermal water loss/proteases. J Invest Dermatol 109: 84–90, 1997***

The outermost layer of the skin, the stratum corneum (SC), is in contact with the environment and hence is susceptible to a wide variety of toxic insults. Injuries to the skin may be induced by contact with solvents or detergents or via mechanical injury such as tape stripping and can disrupt the cutaneous permeability barrier. The cutaneous permeability barrier is localized to the extracellular lamellar membranes of the SC (Elias *et al*, 1993). Acute disruption of the SC barrier by tape stripping, solvent treatment, or detergent treatment elicits a homeostatic response in the epidermis that rapidly restores normal barrier function. This repair response includes the immediate secretion of pre-formed lamellar bodies, an increase in epidermal lipid synthesis, the formation of new lamellar bodies with further secretion and the extracellular remodeling of secreted lamellar-body–derived lipids (Elias *et al*, 1993; Proksch *et al*, 1993). These changes lead to the restoration of lipid-enriched membranes in the SC resulting in normalization of barrier function. The highly predictable and reproducible kinetics of the recovery response can be exploited to (i) link various components of the repair response to a particular phase of the recovery response (Taljebini *et al*, 1996), (ii) elicit previously undetected defects in barrier function, e.g., in chronologic aging (Ghadially *et al*, 1995), or (iii) ascertain whether various topical treatments inhibit or accelerate barrier recovery (Man *et al*, 1993b, 1995b). For example, we have recently reported that certain topically applied physiologic lipid mixtures inhibit barrier recovery (Man *et al*, 1993b), whereas other physiologic and inert lipid mixtures allow normal recovery or even accelerate the repair response (Man *et al*, 1995a, 1996).

Proteolytic enzyme activity has been demonstrated in the SC by

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Abbreviations: t-AMCHA, *trans*-4-(aminomethyl)cyclohexane carboxylic acid; SC, stratum corneum; E64, L-*trans*-epoxysuccinyl-leucylamide-(4-guanidino)-butane; TEWL, transepidermal water loss; TLCK, L-1-chloro-3-(4-tosylamido)-7-amino-2-heptanone hydrochloride; TPCK, L-1-chloro-3-(4-tosylamido)-4-phenyl-2-butanone; uPA, urokinase-type plasminogen activator; PAI, plasminogen activator inhibitor.

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Table I. Effect of Different Classes of Protease Inhibitors on Barrier Recovery[a]

Inhibitor	% Recovery 2 h	4 h	8 h	12 h
Metalloproteases				
Control	28.8 ± 9.0 (NS[b])	50.8 ± 11.6 (NS)	80.7 ± 7.2 (NS)	91.0 ± 3.2 (NS)
EDTA	29.9 ± 10.2	51.6 ± 10.1	84.0 ± 8.0	90.6 ± 7.0
Aspartate proteases				
Control	34.9 ± 7.4 (NS)	49.0 ± 11.1 (NS)	77.1 ± 5.5 (NS)	83.8 ± 3.3 (NS)
Pepstatin	35.9 ± 3.9	44.7 ± 10.4	81.6 ± 8.9	85.8 ± 9.1
Cysteine proteases				
Control	34.0 ± 5.0 (NS)	63.7 ± 5.6 (NS)	70.7 ± 7.0 (NS)	76.4 ± 5.9 (NS)
E64	30.5 ± 4.1	61.8 ± 7.1	66.1 ± 3.8	72.0 ± 5.7
Serine proteases				
Control	35.1 ± 5.6 (NS)	48.4 ± 7.4 ($p < 0.02$)	75.4 ± 6.1 (NS)	84.6 ± 0.8 ($p < 0.05$)
PMSF	44.3 ± 5.6	61.1 ± 3.7	82.2 ± 2.2	90.6 ± 2.3

[a] Serine protease inhibition accelerates barrier recovery in mouse skin after barrier disruption. The flank skin was treated with tape stripping until TEWL reached 8.5 ± 1.5 mg per cm^2 per h. Immediately after barrier disruption 100 μl of a water solution containing 10 mM PMSF or EDTA, 10 μM pepstatin, 1 mM E64 or water alone (control) was applied to the treated area. TEWL was measured at the times indicated after barrier disruption. Values are the mean ± SD (n = 4).

[b] p values are in parentheses and compare control and inhibitor values. NS, not significant.

several laboratories (Hausson *et al*, 1994; Suzuki *et al*, 1994). It has been hypothesized that these proteases are important for the regulated desquamation of the SC and/or the hydrolysis of filaggrin to osmotically active amino acids important for hydration (Scott and Harding, 1986; Hausson *et al*, 1994; Suzuki *et al*, 1994). Wounding the epidermis increases proteolytic activity, which is thought to be important for wound repair (Grondahl-Hausen *et al*, 1988; Romer *et al*, 1991, 1994). Moreover, abnormally high cutaneous proteolytic activity has been observed in several skin diseases associated with abnormal cutaneous barrier function, such as atopic dermatitis, contact dermatitis, and psoriasis (Jensen *et al*, 1988, 1990; Wiedow *et al*, 1992; Spiers *et al*, 1994). Furthermore, psoriatic lesions display increased activity and expression of plasminogen (Jensen *et al*, 1988, 1990; Spiers *et al*, 1994). Because of the increase in proteolytic activity after epidermal injury and the association of increased proteolytic activity with disease states associated with impaired barrier function, we hypothesized that increased protease activity might be detrimental to barrier homeostasis. To test this hypothesis, we compared the effect of various classes of protease inhibitors on the kinetics of barrier recovery after barrier disruption of normal mouse and human skin by tape stripping, acetone treatment, or sodium dodecyl sulfate (SDS) treatment.

MATERIALS AND METHODS

Mouse Studies Male hairless mice (SKH1) were purchased from Charles River (Wilmington, MA). Animals were 7–10 wk of age at the time of study. Phenylmethylsulfonyl fluoride (PMSF), ethylenediamine tetraacetic acid, pepstatin, L-*trans*-epoxysuccinyl-leucylamide-(4-guanidino)-butane (E64), chymostatin, L-1-chloro-3-(4-tosylamido)-4-phenyl-2-butanone (TPCK), trans-4-(aminomethyl)-cyclohexane carboxylic acid (t-AMCHA), aminocaproic acid, and aminobutyric acid were purchased from Sigma (St. Louis, MO). Leupeptin was purchased from Peninsula Laboratories (San Carlos, CA), and L-1-chloro-3-(4-tosylamido)-7-amino-2-heptanone hydrochloride (TLCK) from Fluka (Ronkonkoma, NY).

Barrier disruption was achieved by repeated applications of cellophane tape (3M, St. Paul, MN) on mouse flank skin. Alternatively, the barrier was disrupted with repeated applications of cotton balls soaked with either acetone or 1% SDS for 5 to 10 min. Each procedure was terminated when transepidermal water loss (TEWL) levels reached 8.5 ± 1.5 mg per cm^2 per h (range). Immediately after barrier disruption 100 μl of an aqueous solution containing 10 mM PMSF, ethylenediamine tetraacetic acid, 10 μM pepstatin, 1 mM E62, leupeptin, TLCK, chymostatin, TPCK, 320 mM t-AMCHA (5%), 380 mM aminocaproic acid, or 490 mM aminobutyric acid, or water alone (control) was applied to the treated area. The doses employed for each inhibitor were approximately 10 times the effective concentration required for protease inhibition *in vitro* (Beynon *et al*, 1989). TEWL was measured before barrier disruption, immediately after barrier disruption, and as indicated by using an electrolytic water analyzer (Meeco, Warrington, PA), as described previously (Menon *et al*, 1985; Grubauer *et al*, 1989).

Epidermal hyperplasia was induced by repeated disruption of the barrier by treatment with cellophane tape or acetone twice a day for 7 d as described in detail previously (Denda *et al*, 1996). Briefly, the animals were treated at approximately 8 a.m. and 5 p.m. and each procedure was terminated when TEWL reached 8.5 ± 1.5 mg per cm^2 per h (range). Barrier function did not return to normal between treatments. Just after each treatment 100 μl of a water solution containing 0.064 M t-AMCHA or water alone was applied to the treated area. After the last treatment, the mice were killed and the skin removed for histologic studies.

Human Studies Barrier disruption was achieved by repeated applications of Cellotape (Nichiban, Tokyo, Japan) on the volar forearm of healthy male volunteers until TEWL = 8.5 ± 1.5 mg per cm^2 per h (range). Alternatively, the barrier was disrupted by repeated application of acetone (30–45 min) or 1% SDS (5–10 min). TEWL was measured with an electrolytic water analyzer after barrier disruption at the times indicated.

Microscopy and Morphometrics After fixation with 4% paraformaldehyde, the skin specimens (volar forearm biopsies) were embedded in paraffin and stained with hematoxylin and eosin. Five cross-sections were taken from different parts of each treated area. On each section, 10 points were selected at random; the thickness of the nucleated epidermis was measured with an optical micrometer and the mean value was calculated. Measurements were carried out by an observer who did not know the origin or prior treatment of the sample groups.

Enzyme Localization *In situ* zymography was carried out on skin samples obtained from the inner forearm of healthy males 2 h after tape stripping (TEWL = 10 mg/cm^2/h). Control samples were obtained from an adjacent or contralateral untreated area. Samples were immediately embedded and frozen in O.C.T. (Miles, Elkhart, IN) without fixation. Blocks of embedded tissue were stored at −70°C until sectioned. *In situ* zymography was carried out by a modification of the methods used by Spiers *et al* (1994) and Galis *et al* (1994). Briefly, the overlay solution contained 200 μl of PBS, 266 μl of 2.5% agar solution in water, 80 μl of a solution of purified human plasminogen (0.9 mg per ml), 134 μl of a 1% casein-fluorescein isothiocya-

Table II. Effect of Serine Protease Inhibitors on Barrier Recovery[a]

Inhibitor	% Recovery 2 h	4 h	8 h	12 h
Trypsin-like				
Experiment 1				
Control	7.5 ± 12.1 ($p < 0.03$)[b]	41.3 ± 10.3 ($P < 0.02$)	67.5 ± 6.3 (NS)	72.6 ± 9.7 (NS)
Leupeptin	26.1 ± 4.8	59.1 ± 5.8	73.4 ± 6.5	78.8 ± 3.8
Experiment 2				
Control	20.7 ± 5.5 ($p < 0.03$)	40.5 ± 4.3 (NS)	61.5 ± 3.9 ($p < 0.006$)	74.8 ± 2.6 (NS)
TLCK	23.9 ± 2.2	49.9 ± 10.0	74.2 ± 4.8	82.2 ± 4.4
Chymotrypsin-like				
Experiment 3				
Control	16.6 ± 7.8 (NS)	37.1 ± 16.6 (NS)	67.9 ± 6.1 (NS)	81.1 ± 2.2 (NS)
Chymostatin	14.9 ± 6.7	38.8 ± 10.5	63.5 ± 4.9	82.5 ± 1.0
Experiment 4				
Control	33.6 ± 5.8 (NS)	50.3 ± 10.5 (NS)	79.7 ± 3.1 (NS)	82.9 ± 5.5 (NS)
TPCK	35.3 ± 5.5	52.5 ± 8.3	74.3 ± 6.4	85.1 ± 5.8

[a] Trypsin-like serine protease inhibition accelerates barrier recovery in mouse skin after barrier disruption. The flank skin was treated with tape stripping until TEWL reached 8.5 ± 1.5 mg per cm^2 per h. Immediately after barrier disruption 100 μl of a water solution containing 1 mM leupeptin, TLCK, chymostatin, TPCK, or water alone (control) was applied to the treated area. TEWL was measured at the times indicated after barrier disruption. Values are mean ± SD (n = 4).

[b] p values are in parentheses and compare control and inhibitor values. NS, not significant.

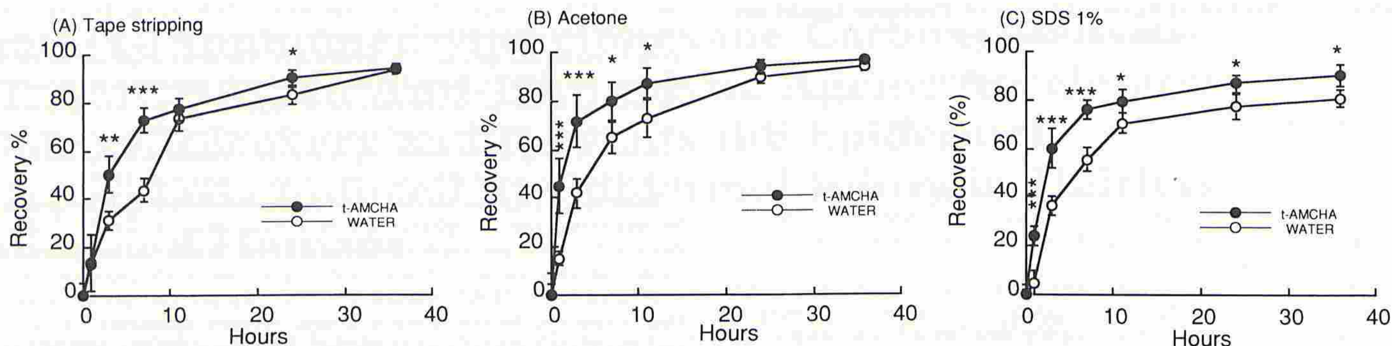

Figure 1. t-AMCHA accelerates barrier recovery in mouse skin after tape stripping *(A)*, acetone treatment *(B)*, or SDS treatment *(C)*. The flank skin was treated until TEWL reached 8.5 ± 1.5 mg per cm^2 per h. Immediately after barrier disruption 100 μl of t-AMCHA solution (0.32 M) or water alone (control) was applied to the treated area. TEWL was measured at the times indicated after barrier disruption. *Error bars*, SD (n = 4). $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.005$.

nate solution in phosphate-buffered saline, and 2.4 μl of 10% sodium azide. This overlay mixture was prepared at 50°C, and 50 μl of the mixture was applied to prewarmed 10-μm-thick cryostat sections mounted on Superfrost slides and spread evenly under glass coverslips (18 × 18 mm). Slides were incubated at 37°C in humid chambers for 3 d, and the lysis of the substrate was determined by examination with a fluorescent microscope (Olympus, AH3-RFC, Tokyo, Japan). Loss of fluorescence is indicative of proteolysis.

Data Analysis The barrier recovery results are expressed as percent recovery because of variations from day to day in the extent of disruption of the barrier. In each animal the percent recovery was calculated by the following formula: 1 − [(TEWL immediately after treatment − TEWL at indicated time)/(TEWL immediately after treatment − baseline TEWL)] × 100%. Results are expressed as the mean ± SD. Statistical differences were determined by a two-tailed Student's t test because all data were compared to simultaneously studied controls.

RESULTS

Our initial experiments tested the ability of different classes of protease inhibitors to modulate the kinetics of barrier recovery after acute cutaneous injuries that disrupt the permeability barrier. At the doses employed, ethylenediamine tetraacetic acid (a metalloprotease inhibitor), pepstatin (an aspartic protease inhibitor), or E64 (a cysteine protease inhibitor) did not altered the kinetics of barrier recovery **(Table I)**. In contrast, treatment with PMSF, a general serine protease inhibitor, significantly accelerated barrier recovery at 4 and 12 h.

Serine protease inhibitors can be divided into two general classes: trypsin-like and chymotrypsin-like. Trypsin-like serine protease inhibitors (leupeptin and TLCK) significantly accelerated barrier recovery, whereas chymotrypsin-like serine protease inhibitors [chymostatin and TPCK had little or no effect on barrier recovery **(Table II)**.

We next tested the effects of of t-AMCHA, a trypsin-like protease inhibitor that specifically inhibits the formation of plasmin, an extracellular serine protease (Reinartz *et al*, 1993). As shown in **Fig 1**, topical treatment with t-AMCHA significantly accelerates barrier recovery after tape stripping **(Fig 1*A***, 66% faster at 4 h), acetone treatment **(Fig 1*B***, 140% and 75% faster at 2 and 4 h, respectively), or SDS treatment **(Fig 1*C***, 11-fold and 58% faster at 2 and 4 h, respectively). In contrast, minimally active structural analogs of t-AMCHA (aminocaproic acid and aminobutyric acid) either delay or do not alter the kinetics of barrier repair **(Fig 2)**.

We next determined whether t-AMCHA would also influence the kinetics of barrier recovery in humans. As shown in **Fig 3**, after tape stripping, acetone treatment, or SDS treatment, t-AMCHA markedly accelerate barrier recovery, a result similar to that observed in mice after barrier disruption.

In recent studies, we demonstrated that repeated disruption of the barrier by either tape stripping or acetone treatment for 7 d results in cutaneous pathologic changes, including epidermal hyperplasia, and that these changes are linked to epidermal injury rather than barrier disruption (Denda *et al*, 1996). Therefore, we next asked whether t-AMCHA also would impede the development of epidermal hyperplasia. As shown in **Figs 4** and **5**, t-AMCHA treatment greatly decreased the epidermal hyperplasia induced by either repeated tape stripping or acetone treatment. These results show that protease activation contributes to the development of epidermal hyperplasia after stratum corneum injury.

Because the above studies show that plasmin-type serine proteases are detrimental to barrier homeostasis and potentially contribute to cutaneous pathology, we next asked whether injuries to the epidermis that result in barrier disruption stimulate epidermal proteolytic activity. As shown in **Fig 6**, tape stripping induces epidermal proteolytic activity. This epidermal proteolytic activity

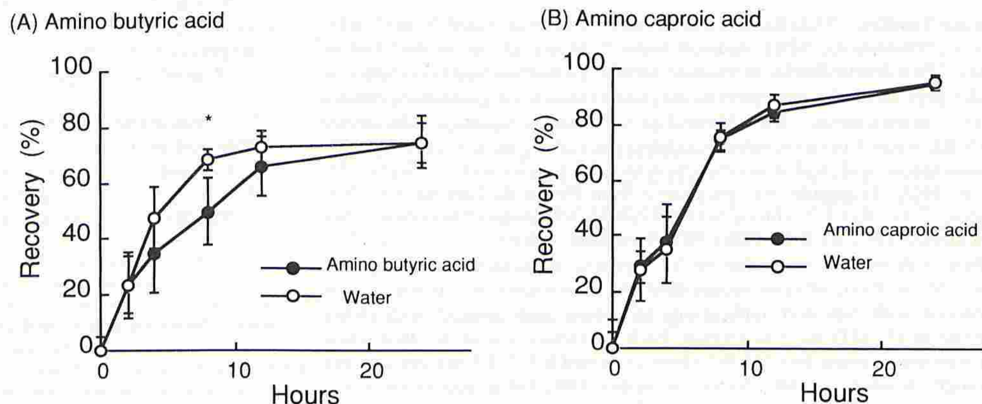

Figure 2. Analogs of t-AMCHA either delay or do not alter the kinetics of barrier repair. Mouse flank skin was treated with tape stripping until TEWL reached 8.5 ± 1.5 mg per cm^2 per h. Immediately after tape stripping 100 μl of aminobutyric acid solution (0.49 M) *(A)*, aminocaproic acid (0.38 M) *(B)*, or water alone (control) was applied to the treated area. TEWL was measured at the times indicated after barrier disruption. *Error bars*, SD (n = 4). $^*p < 0.05$.

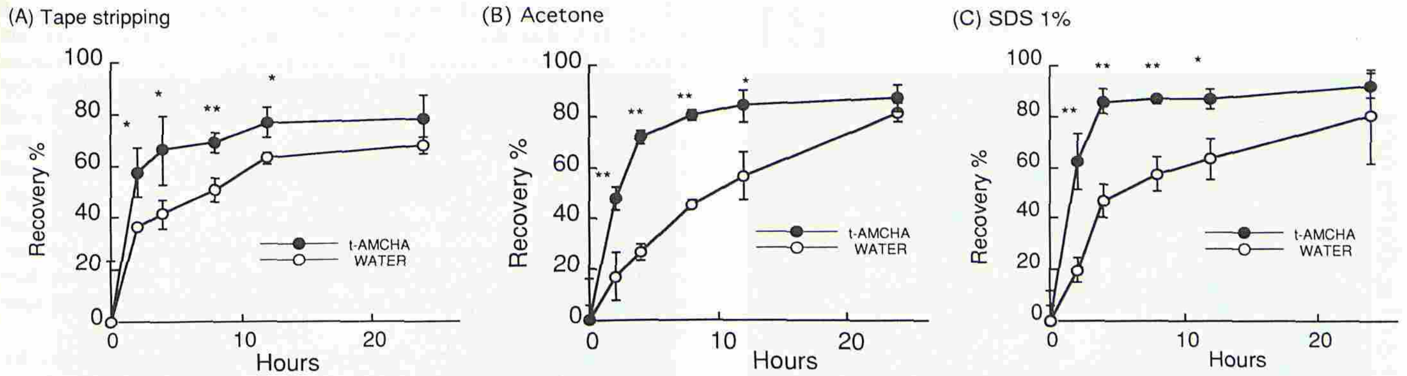

Figure 3. t-AMCHA accelerates barrier recovery in human skin after tape stripping *(A)*, acetone treatment *(B)*, or SDS treatment *(C)*. Forearm skin was treated until TEWL reached 8.5 ± 1.5 mg per cm^2 per h. Immediately after barrier disruption 100 μl of t-AMCHA solution (0.32 M) or water alone (control) was applied to the treated area. TEWL was measured at the times indicated after barrier disruption. *Error bars,* SD (n = 3). *$p < 0.05$; **$p < 0.01$.

was decreased in plasminogen-free substrate gel, suggesting that a major portion of the increase in proteolytic activity in the epidermis after tape stripping occurred via the plasminogen/plasmin system. Most importantly, the increase in epidermal proteolytic activity induced by tape stripping could be markedly inhibited by t-AMCHA even in the presence of plasminogen. These results suggest an important role for the plasminogen/plasmin system in the increased epidermal proteolysis that occurs after barrier disruption. Further, they correlate with the ability of plasminogen/plasmin inhibitors to accelerate barrier recovery.

Figure 4. t-AMCHA treatment decreases epidermal hyperplasia induced by either repeated tape stripping or acetone treatment of mouse flank skin. Acetone or tape stripping was carried out twice a day for 7 d until the TEWL levels reached 8.5 ± 1.5 mg per cm^2 per h. Just after each treatment, 100 μl of a water solution containing 0.064 M t-AMCHA or water alone was applied to the treated area. Skin samples were fixed and stained with hematoxylin and eosin. After either repeated tape stripping *(A)* or acetone *(B)* epidermal hyperplasia is present in contrast to untreated control *(E)*. t-AMCHA treatment of both tape-stripped *(C)* or acetone-treated skin *(D)* results in a decrease in epidermal hyperplasia. *Scale bar,* 40 μm.

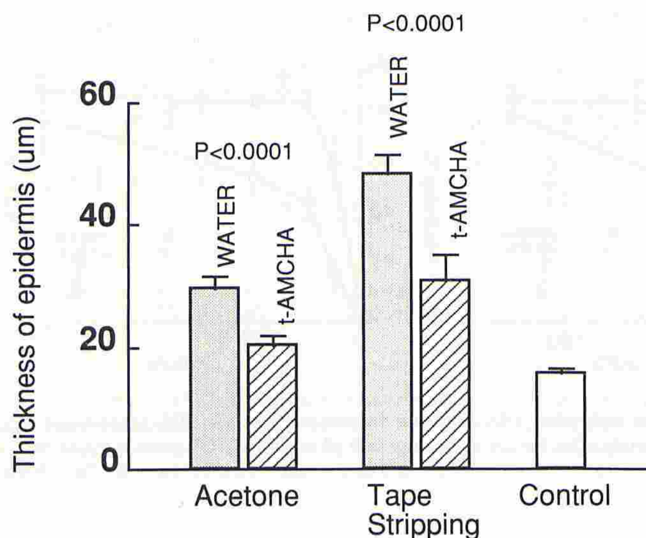

Figure 5. t-AMCHA treatment decreases epidermal hyperplasia induced by either repeated tape stripping or acetone treatment of mouse flank skin. Acetone or tape stripping was carried out twice a day for 7 d until the TEWL levels reached 8.5 ± 1.5 mg per cm^2 per h. Five sections were taken from different parts of each treated area. On each section, 10 points were selected at random. The thickness of the nucleated epidermis was measured with an optical micrometer, and the mean value was calculated. Measurements were carried out in an observer blinded fashion. *Error bars,* SD (n = 5).

DISCUSSION

Previous studies from our laboratory have shown that injuries to the epidermis that disrupt the cutaneous permeability barrier result in a homeostatic recovery response that rapidly restores barrier function toward normal (Elias *et al*, 1993; Proksch *et al*, 1993). Over the years, we have identified a number of metabolic responses that occur in the underlying epidermis that are important for barrier homeostasis. As one would expect, inhibition of each of these metabolic processes delays barrier recovery. For example, inhibition of epidermal lipid synthesis, lamellar body secretion, or extracellular processing delays barrier repair (Feingold *et al*, 1990; Holleran *et al*, 1991, 1993; Man *et al*, 1993a, 1995a, 1995b).

In the current study, we provide evidence that endogenous proteolytic activity, which increases as a consequence of epidermal injury, may retard the rate of barrier recovery. By using *in situ* zymography, we demonstrated in humans that tape stripping increases proteolytic activity in the epidermis. Similar results were observed in mice after either tape stripping or acetone treatment (data not shown). This increase in epidermal proteolytic activity required plasminogen and was markedly inhibited by t-AMCHA, suggesting that the plasminogen/plasmin system accounts for a large part of the increase in epidermal proteolytic activity that was induced by barrier disruption. Other investigators have shown that wounding the epidermis also activates the plasminogen/plasmin system (Grondahl-Hansen *et al*, 1988; Romer *et al*, 1991, 1994).

Several different types of protease inhibitors, including metallo-, aspartic, and cysteine protease inhibitors, had no beneficial effects on barrier recovery. In contrast, topical application of several different serine protease inhibitors accelerate barrier recovery. Specifically, trypsin-like serine protease inhibitors improve barrier recovery whereas chymotrypsin-like serine protease inhibitors had no effect. Moreover, t-AMCHA, a serine protease inhibitor that prevents the activation of plasminogen by inhibiting binding to cell surface receptors (Reinartz *et al*, 1993), also enhances barrier recovery. Furthermore, t-AMCHA accelerates barrier recovery in both mice and humans after several unrelated insults, such as tape stripping, solvent treatment (acetone), or detergent treatment (1% SDS). In contrast, chemically related but inactive analogues of t-AMCHA that do not inhibit plasminogen activation have no effect on barrier recovery. These results suggest that the activation of the plasminogen to plasmin, a serine protease, is detrimental for barrier homeostasis. The abnormalities in barrier function reported in atopic dermatitis, contact dermatitis, and psoriasis (Grice, 1980; Pinnagoda *et al*, 1989; Marks *et al*, 1990; Halkier-Sorensen and Thastrup-Pedersen, 1991) could be related to the abnormally high proteolytic activity observed in these disorders. Further, our studies indicate that serine protease inhibitors, particularly of the plasminogen/plasmin system, can have beneficial effects on barrier homeostasis in both mice and humans after a variety of different insults.

Previous studies have demonstrated that repeated insults by treatment with acetone or tape stripping produces epidermal hyperplasia (Denda *et al*, 1996). Moreover, the magnitude of the epidermal hyperplasia was directly correlated with both the degree and the duration of barrier disruption (Denda *et al*, 1996). Occlusion with a water-impermeable membrane, however, did not prevent the epidermal hyperplasia, indicating that barrier disruption was not the cause of these pathologic changes but, rather, was a marker for the degree of injury (Denda *et al*, 1996). In the current study, we demonstrate that t-AMCHA treatment decreases the epidermal hyperplasia that occurs after repeated injury to the stratum corneum. Furthermore, previous studies have demonstrated that topical t-AMCHA reduces the hyperplastic dry scaly skin that is induced by SDS treatment (Kitamura *et al*, 1995). This indicates that t-AMCHA not only accelerates barrier repair but also provides other potential benefits after epidermal injuries.

Normal epidermis contains many of the components of the plasminogen system (Kramer *et al*, 1995). Urokinase-type plasminogen activator (uPA) is the predominant plasminogen activator in normal epidermis, but in disease states such as psoriasis, the quantity of tissue-type plasminogen activator increases (Jensen *et al*, 1988; Spiers *et al*, 1994). Natural inhibitors of uPA and tissue-type plasminogen activator, PAI-1 and PAI-2, are also made by keratinocytes (Hashimoto *et al*, 1989; Romer *et al*, 1991). When the balance of activation versus inhibition results in the activation of plasminogen, this leads to the conversion of plasminogen to plasmin. Plasmin is a serine protease that can hydrolyze a wide variety of extracellular proteins, including other proteases leading to their activation (Vassalli *et al*, 1991). Thus, plasminogen activation leads to a cascade of proteolytic events that may effect a large number of extracellular proteins thereby altering cell function.

The importance of the plasminogen plasmin cascade in epidermal biology and pathophysiology has been suggested by a large number of studies (Kramer *et al*, 1995). After wounding the epidermis, there is an increase in uPA and its receptor on keratinocyte outgrowths, suggesting that the focal formation of plasmin and increased local extracellular proteolysis may be important in keratinocyte migration (Grondahl-Hansen *et al*, 1988; Romer *et al*, 1991, 1994). Targeted disruption of the plasminogen gene results in impaired wound healing (Romer *et al*, 1996). In several disease states, such as psoriasis and pemphigus, the expression of components of the plasminogen activation system are increased, suggesting that increased plasmin activity may contribute to disease manifestations (Jensen *et al*, 1988, 1990; Baird *et al*, 1990; Spiers *et al*, 1994). Moreover, the administration of plasmin to skin organ cultures induces acantholysis and the ability of pemphigus IgG to induce acantholysis is blocked by PAI-2, an inhibitor of plasminogen activation (Hunziker and Vassalli, 1987; Spiers *et al*, 1994). Lastly, the overexpression of human PAI-1 in transgenic mice results in a greatly thickened stratum corneum, suggesting a role for plasmin in epidermal desquamation (Lyons-Giordano and Lazarus, 1995). Along similar lines in keratinocytes grown in the absence of thyroid hormone, plasminogen activator activity is decreased 70–80% and there is a decreased shedding of cornified cells, but conversely, the addition of retinoic acid increases both uPA and tissue-type plasminogen activator activity and decreases cell cohesion (Isseroff *et al*, 1989). Thus, there are many avenues by which the activation of the

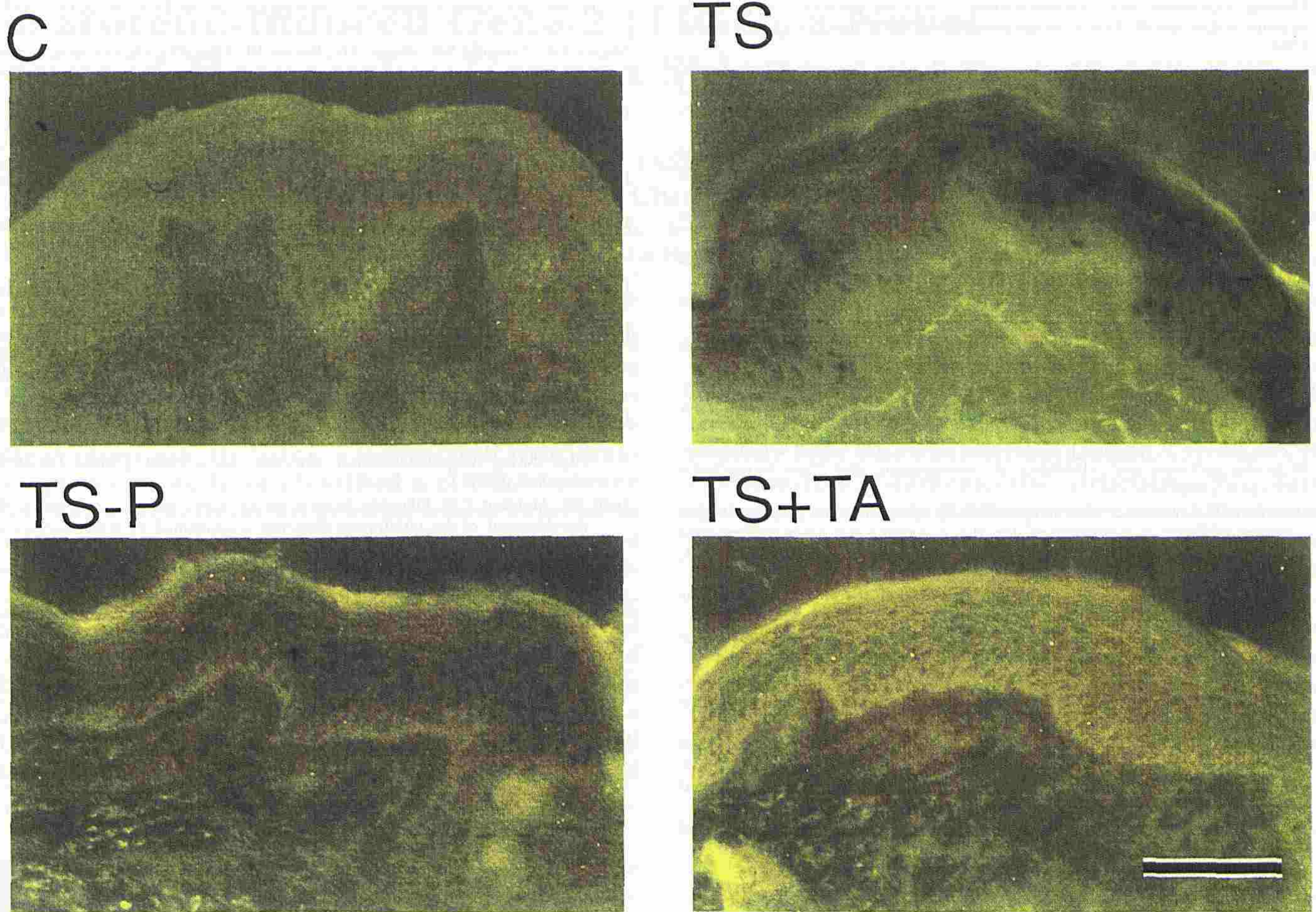

Figure 6. Barrier disruption with tape stripping induces epidermal proteolytic activity. Human forearm skin was treated until TEWL reached 10 mg per cm^2 per h. Proteolytic activity is barely detectable in the epidermis of untreated control skin (C). Two hours after tape stripping, the proteolytic activity increased in the epidermis (disappearance of light green color over epidermis) (TS). This epidermal proteolytic activity was decreased in plasminogen-free substrate gel (TS-P) and was totally inhibited by t-AMCHA (TS+TA) even in the presence of plasminogen. *Scale bar,* 100 μm.

plasminogen plasmin system could potentially alter epidermal function.

We have demonstrated in both mice and humans that the topical application of t-AMCHA, which inhibits plasminogen activation, accelerated barrier recovery after barrier disruption by acetone treatment, SDS treatment, or tape stripping. Moreover, the epidermal hyperplasia that is induced by repeated epidermal injuries by either acetone treatment or tape stripping is decreased by topical t-AMCHA treatment. These results suggest that manipulations that injure the stratum corneum activates the plasminogen/plasmin system. This increase in extracellular protease activity is detrimental to barrier repair and may induce pathologic changes in the skin. Thus, a new potential strategy from improving barrier repair and decreasing the pathology associated with cutaneous injury may be to inhibit the activation of plasminogen or the activity of plasmin.

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