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Levels of Skin-Derived Antileukoproteinase (SKALP)/Elafin in Serum Correlate with Disease Activity During Treatment of Severe Psoriasis with Cyclosporin A

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The epidermal serine proteinase inhibitor SKALP (also known as elafin), directed against human leukocyte elastase and proteinase 3, is strongly induced in suprabasal keratinocytes during inflammation. The presence of SKALP/elafin in urine has been demonstrated for several inflammatory skin disorders, such as psoriasis, erythroderma, and erysipelas. In this study we investigated whether SKALP/elafin levels in serum and urine of psoriatic patients can be used as a marker for disease activity during treatment. Patients with severe chronic disabling psoriasis were treated for 16 weeks with cyclosporin A, which resulted in a marked clinical improvement as measured with the PASI score. SKALP/elafin levels both in serum and urine were determined with an enzyme-linked immunoabsorbent assay (ELISA). Measurements were performed at the start of the cyclosporin A treatment, and after regular intervals up to 16 weeks. The results indicate that 1) SKALP/elafin determination in serum rather than in urine is the preferred method, because the decrease in serum SKALP levels during therapy is more pronounced and correlated better with the clinical course of the patients; 2) SKALP/elafin levels in serum decreased during cyclosporin A treatment (p < 0.05); and 3) SKALP/elafin levels in serum correlate with the PASI score (p < 0.01). We conclude that SKALP/elafin measurement in serum of patients with severe psoriasis provides a tool for monitoring disease activity. Keywords: ELISA, proteinase inhibitor, epidermis. J Invest Dermatol 104:189-193, 1995

We have recently shown that skin-derived antileukoproteinase (SKALP), a serine proteinase inhibitor virtually absent in normal epidermis, is induced in lesional psoriatic skin [1]. We found that SKALP, also known as elafin [2,3], is not specific for psoriasis, but can be considered as one of the markers of the regenerative differentiation program that is also found in other conditions such as wound healing [4,5] and several inflammatory skin diseases [6]. SKALP/elafin is a strong inhibitor of the leukocytic enzymes elastase [1] and proteinase-3 [7], and is putatively involved in the regulation of cutaneous inflammation. In previous studies SKALP/elafin was described biochemically [8] and characterized at the protein and DNA level [9]. We established the cellular source and localization in psoriatic epidermis [10], and we found that SKALP/elafin is differentially expressed in human epidermal tumors [11]. Recently we have been able to assign the SKALP/elafin gene to chromosome region 20q12-q13 [12]. The gene has been given the approved name of Protease Inhibitor, skin-derived (SKALP) and the symbol PI3 in the Genome Data Base of the HUGO nomenclature committee.

In psoriasis, SKALP/elafin is expressed in the upper spinous layers of lesional skin [10]. We showed that SKALP/elafin as expressed in cultured epidermal keratinocytes is translated as a 12.3-kDa protein. Cleavage of the signal peptide yields a 9.9-kDa protein that is the major form found in cultured cells, as was confirmed by purification and N-terminal amino acid sequencing [9]. The presence of a signal peptide suggests that SKALP/elafin is a secreted protein. We indeed found that significant amounts of biologically active SKALP/elafin can be found in the urine of psoriatic patients [13], a finding that was recently confirmed by others [14]. In urine of healthy individuals SKALP/elafin was not detectable. Apparently, once secreted in the epidermis, SKALP/elafin is able to reach the circulation, and is subsequently cleared via the kidney. Although we were then not able to measure SKALP/elafin in serum, this was a likely route, because SKALP/elafin is a low-molecular-weight cathepic protein that will easily cross basal membranes.

In the present study there were two objectives: first, we wanted to develop an assay for SKALP/elafin assessment in serum of psoriatic patients, and second we wanted to investigate if measurement of SKALP/elafin in urine and serum could be used as a marker for disease activity during therapy. In our previous study, we used a functional biochemical assay to measure SKALP/elafin in urine [13]. However, in serum this is not possible due to interfering factors. We therefore developed an enzyme-linked immunosorbent assay (ELISA) that allows measurement of SKALP/elafin both in serum and in urine. Using this ELISA we found that patients with severe psoriasis during therapy with cyclosporin A (CyA) showed a decrease of SKALP/elafin activity in serum that correlated with a decrease in clinical scores (psoriasis area and severity index (PASI) score). These findings suggest that SKALP/elafin can be used as a
marker for disease activity and can be used as a quantitative measure to monitor therapeutic effects.

MATERIALS AND METHODS

Chemicals  Tween-20 was obtained from Merck, Schuchardt, Hohenbrunn bei Munich, Germany, and bovine serum albumin (BSA) from Organon Teknika, Boxtel, The Netherlands. Swine-anti-rabbit peroxidase was obtained from Dako, Copenhagen, Denmark, and o-phenylenediamine. 2HCl from Pierce, Rockford, IL. Microtiter plates (96 cells) for ELISA were obtained from Greiner, Alphen a/d Rijn, The Netherlands. Recombinant SKALP/elaﬁn was a kind gift from Dr. Norman Russell, ICI Pharmaceuticals, UK.

Subjects, Sample Collection and Preparation Fifteen healthy volunteers (5 male, 10 female, ages 2—79 years) took part in the experiments to study age or sex differences in SKALP/elaﬁn serum levels. For determination of circadian variation blood was drawn every 4 h during 24 h in a psoriatic patient and a healthy control. Six male patients (ages 36—64 years, mean age 48 years) with severe chronic disabling psoriasis participated. Patients had not used any systemic treatment for at least 4 weeks before the start of the study. Using routine blood tests for liver and kidney functions, no side effects on these organs could be demonstrated; one patient showed hypertrichosis. Urine and blood samples of one of the patients could not be taken until 2 weeks after the start of the CyA treatment.

Sensitivity and Accuracy of the ELISA The ELISA we developed is of the competitive type, based on the displacement of a defined amount of recombinant SKALP/elaﬁn by SKALP/elaﬁn in the urine or serum samples. A calibration curve from which the sample values are read in the interval between 3—40 ng per ml is given in Fig 1. The detection limit is 3 ng/ml.

Experiments to check the recovery of recombinant SKALP/elaﬁn added to normal human serum revealed that recovery is temperature dependent (not shown). When the ELISA was performed at 4°C a recovery of 100% was found (Fig 2). Figure 2 also shows that a pool of normal human serum has a background level of SKALP/elaﬁn, which appeared to be about 9 ng/ml (intercept of the Y-axis). The standard deviation of measurements in quadruplicate was within 10%.

SKALP/elaﬁn Measurement in Urine SKALP/elaﬁn levels were expressed in ng/ml urine, and corrections for creatinine concentrations were made. SKALP/elaﬁn levels in urine varied from 3 ng/ml up to more than 120 ng/ml (Fig 3). Five of six patients showed a clear decrease in urinary SKALP/elaﬁn levels. One patient showed extremely low values of creatinine concentration, which had an enormous impact on correction for creatinine (Fig 3); note that his serum level of SKALP/elaﬁn decreased during treatment (Fig 4). Decrease of SKALP/elaﬁn levels in urine during treatment was statistically signiﬁcant (Fisher-z, p < 0.05).

Urinary SKALP/elaﬁn levels decreased, but were not found below 3 ng/ml. Healthy controls show a similar level of SKALP/elaﬁn in urine (data not shown), and 3—10 ng/ml may be regarded as a normal background level of SKALP/elaﬁn in human urine.

SKALP/elaﬁn Measurement in Serum Serum levels for SKALP/elaﬁn were measured in 15 healthy volunteers. No effect of age on SKALP/elaﬁn levels was found (not shown). A small difference in SKALP/elaﬁn serum levels (ng/ml) was found between male (14.6 ± 2.6) and female (8.5 ± 6.1). No circadian rhythm was observed (not shown). SKALP/elaﬁn levels in psoriatic patients varied from 11 ng/ml up to more than 300 ng/ml (Fig 4). These levels significantly decreased during treatment with CyA (Fisher-z, p < 0.05) and correlated well with clinical improvement as expressed in the PASI score (Fisher-z, p < 0.01). This decrease in SKALP/elaﬁn level was found in the serum of all six patients. SKALP/elaﬁn levels in serum were about sixfold higher than in urine, and levels in serum were positively correlated with levels in urine (Fisher-z, p < 0.01).

RESULTS

PASI Score and CyA Treatment All patients showed good clinical improvement during 16 weeks of treatment with CyA, and apart from slight gastrointestinal discomfort no complaints were reported. Mean PASI score was 20.0 (10.6—36.0) at the start of the treatment, and decreased to 3.4 (2.0—5.4) at week 16. In two patients dosage of CyA was increased at week 8 due to lack of clinical progress. Using routine blood tests for liver and kidney functions, no side effects on these organs could be demonstrated; one patient showed hypertrichosis. Urine and blood samples of one of the patients could not be taken until 2 weeks after the start of the CyA treatment.

Figure 1. Calibration curve of recombinant SKALP/elaﬁn. A sigmoid relationship between the ELISA signal and the SKALP/elaﬁn concentration of the samples is found. The useful range for measurement of SKALP/elaﬁn concentrations is between 3 and 40 ng/ml.
SKALP/ELAFIN LEVELS IN SERUM

DISCUSSION

Increased amounts of SKALP/elafin have been demonstrated in urine of patients with psoriasis [13] and several other inflammatory skin diseases [14]. Because SKALP/elafin is barely detectable in normal skin and is strongly expressed in inflamed skin, we hypothesized that the increase in urinary SKALP/elafin in psoriatics is derived from local production in skin. This would be in line with the fact that SKALP/elafin is a secreted molecule [16] and the notion that epidermis acts as a secretory organ as recently described [17,18]. However, no definitive proof could be given for the epidermal origin of urinary SKALP/elafin in psoriatic patients, because SKALP/elafin could not be demonstrated in the intermediate compartments between epidermis and urine (i.e., dermis and blood). In our initial study we used a functional assay to measure SKALP/elafin, that could not be applied to serum, due to interference by the overwhelming amount of other plasma-derived proteinase inhibitors.

Intraepidermal accumulation of PMN is an early event in the pathogenesis of the psoriatic lesion [19], and PMN-derived proteinases were shown to degrade the dermo-epidermal junction in vitro [20]. This suggests that elastase may be involved in PMN migration and PMN-dependent tissue damage and may promote the inflammatory response. Several antipsoriatic therapies are known to interfere with this PMN migration, suggesting that they are involved in the downregulation of inflammation [21]. In addition to proteinases from inflammatory cells, keratinocyte-derived proteinases such as plasminogen activator may act as a pro-inflammatory mediator in psoriatic skin [22]. Synthesis of proteinase inhibitors that counteract proteinases by forming inactive complexes is one of the in vivo mechanisms to protect tissue against unwanted proteolysis [23]. These proteinase inhibitors may be present in plasma, or can be produced locally at the site of action [8,24,25]. Complexes between elastase and elastase inhibitors in serum, sputum, bronchoalveolar lavage, and urine have been described, the inhibitors being α1-PI [26–28], antileukoproteinase [29], or SKALP/elafin [13]. Levels of these complexes may be related to disease activity, and measurement may provide a tool for monitoring therapeutic result. Increased levels of complexes between elastase and inhibitors, but also of free elastase or free inhibitor, have been reported in lung cancer [30], neonatal systemic infection [31,32], adult respiratory distress syndrome [27,32], cervical cancer [33,34], psoriasis, eczema, and other cutaneous disor-

![Figure 2](image-url) Mean values ± 2 SD of measurements in quadruplicate of three concentrations of recombinant SKALP/elafin in normal human serum. SD is 10%. The intercept with the Y-axis indicates that in humans a background serum level of SKALP/elafin appears to be present. This background level is about 9 ng/ml.

![Figure 3](image-url) SKALP/elafin levels in urine of six psoriatic patients (a–f) during treatment with CyA. SKALP/elafin levels (ng/ml) are shown before (open squares) and after correction for creatinine concentration (solid squares). Decrease of SKALP/elafin levels in urine is statistically significant (Fisher z, p < 0.05).
Figure 4. SKALP/elafin levels in serum of six psoriatic patients (a-f) during treatment with CyA. Amount of SKALP/elafin in serum is given in ng/ml (solid squares). Decrease of SKALP/elafin levels in serum is statistically significant (Fisher z, p < 0.05). PASI scores at time of sampling are given (open squares), and these levels can be used as parameters for monitoring disease activity.

In normal epidermis, anti-elastase activity is hardly detectable [1], although the presence of α1-PI and α2 macroglobulin in normal skin has been described [38,39]. Teleologically, it is plausible that after a disturbance of the proteinase-antiproteinase balance in skin, resulting in an excess of elastase, SKALP/elafin is produced to limit proteolytic action of this leukocytic proteinase. After a period of proteolysis by elastase a rebalancing takes place, and SKALP/elafin molecules may temporarily outnumber elastase molecules. Complexes are secreted in urine [13], but epidermic clearance via loss of scales that contain complexes may take place as well. Finally, restoration of normal homeostasis is followed by an off-switch of SKALP/elafin production by the epidermal cells.

Using a competitive-type ELISA, we were able to demonstrate the presence of SKALP/elafin in serum of normal individuals and psoriatic patients. The assay was found to be reproducible and reasonably sensitive (detection limit 3 ng/ml). We found a background level of SKALP/elafin in serum of healthy controls and symptom-free psoriatic patients that is probably derived from the turnover in tissues where SKALP/elafin is constitutively expressed. We have recently completed a survey of SKALP/elafin in normal human epithelia (Alkemade et al, manuscript in preparation) and found that the molecule is expressed in a limited number of normal tissues. This background level of SKALP/elafin in serum negatively influences the sensitivity of the assay for detecting an increase in inflammatory skin diseases.

In severe psoriatic patients with a large area of involved skin, serum levels are significantly higher than in healthy controls and symptom-free psoriatic patients. It is shown that during therapy with CyA, which is a potent antipsoriatic drug, serum levels gradually decrease and stabilize to a level that is still higher than in normal controls. This decrease in SKALP/elafin levels correlates with the decrease in PASI scores, and we assume that the decrease in serum SKALP/elafin levels during therapy reflects the rate of normalization of the epidermis. The SKALP/elafin concentrations in serum were about sixfold higher than in urine, and the decrease in serum during treatment was more impressive and correlated better with the clinical course. Therefore, determination of SKALP/elafin in serum rather than in urine was considered to be the method of preference. Because SKALP/elafin levels correlated well with the clinical state as represented by the PASI score, we conclude that SKALP/elafin measurement in serum of patients with severe psoriasis provides a tool for monitoring disease activity.

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REFERENCES


