Endothelins (ETs), in addition to their physiological functions, exert important actions at the skin level, such as increase of keratinocyte proliferation, neo-angiogenesis and leukocyte chemotaxis, which are among the main characteristics of psoriasis. To assess a possible ET-1 involvement in plaque-type psoriasis, ET-1 determinations were carried out in 15 sera and 8 lesional skin samples with normal skin. Immunohistochemistry revealed a specific ET-1 expression in keratinocytes (7). Moreover, ET-1 could be involved in the pathogenesis of psoriasis (13) and to share some biological functions with ET-1, such as keratinocyte proliferation and leukocyte chemotaxis (14, 15). In the present study, we evaluated the serum ET-1 levels in 15 psoriatic patients, compared to skin extracts from normal individuals. In addition, since interleukin-8 (IL-8) has been reported to be involved in the pathogenesis of psoriasis (13) and to share some biological functions with ET-1, such as keratinocyte growth, vascular proliferation and leukocyte chemotaxis (14, 15), we also evaluated the levels of this cytokine in extracts of lesional and non-lesional psoriatic skin to verify possible quantitative correlations.

The findings shown in this work may be useful to highlight a possible ET-1 involvement in the inflammatory processes associated with plaque-type psoriasis.

MATERIALS AND METHODS

Serum samples were obtained from 15 patients affected with active plaque-type psoriasis (4 males and 11 females, median age: 53 years, range 31–75).

The disease severity was evaluated by means of the Psoriasis Area

© 1998 Scandinavian University Press. ISSN 0001-5555 Acta Derm Venereol (Stockh) 78
and Severity Index (PASI). In our patients the median PASI score was 12.4, ranging from 6.3 to 31.7.

None of the patients had received topical or systemic antipsoriatic treatments for at least 10 days before enrollment. Cyclosporin A had not been used in these patients during the previous cycle of therapy, excluding a possible induction of ET synthesis by this drug (15).

Other concomitant diseases were excluded by means of a complete clinical and laboratory examination. After blood collection, sera were stored at −80 °C until used.

In 8 (4 males and 4 females; median age: 48 years 34–70; median PASI score: 14.5, range 6.3–31.7) of the 15 patients, 6-mm diagnostic punch biopsies were also performed, under local anaesthesia with minimum 0.4 pg/ml (median 0.9 pg/ml; minimum 0.4 pg/ml; maximum 1.7 pg/ml) as compared with the control group (median 0.6 pg/ml; minimum under detection limit; maximum 0.8 pg/ml) (p = 0.04).

In addition, to verify whether the patient ET-1 values were related to the disease severity, a correlation test was performed on the patients' data. As shown in Fig. 1A, a significant correlation between serum ET-1 levels and PASI scores was observed (r = 0.60; p = 0.02).

**ET-1 in sera**

To compare serum ET-1 levels of psoriatic and normal subjects, 15 serum samples from the patients and 15 from the controls were assayed using ELISA techniques. The serum median ET-1 levels were increased in the patients (median 0.9 pg/ml; minimum 0.4 pg/ml; maximum 1.7 pg/ml) as compared with the control group (median 0.6 pg/ml; minimum under detection limit; maximum 0.8 pg/ml) (p = 0.04).

**ET-1 in skin extracts**

Other experiments were undertaken to investigate whether the difference observed at the systemic level could also be revealed parametric tests were used: the Wilcoxon rank test, the Mann–Whitney test and, finally, the Spearman's rank correlation test.

**RESULTS**

**ET-1 in sera**

To compare serum ET-1 levels of psoriatic and normal subjects, 15 serum samples from the patients and 15 from the controls were assayed using ELISA techniques. The serum median ET-1 levels were increased in the patients (median 0.9 pg/ml; minimum 0.4 pg/ml; maximum 1.7 pg/ml) as compared with the control group (median 0.6 pg/ml; minimum under detection limit; maximum 0.8 pg/ml) (p = 0.04).

In addition, to verify whether the patient ET-1 values were related to the disease severity, a correlation test was performed on the patients' data. As shown in Fig. 1A, a significant correlation between serum ET-1 levels and PASI scores was observed (r = 0.60; p = 0.02).

**ET-1 in skin extracts**

Other experiments were undertaken to investigate whether the difference observed at the systemic level could also be revealed parametric tests were used: the Wilcoxon rank test, the Mann–Whitney test and, finally, the Spearman's rank correlation test.

**RESULTS**

**ET-1 in sera**

To compare serum ET-1 levels of psoriatic and normal subjects, 15 serum samples from the patients and 15 from the controls were assayed using ELISA techniques. The serum median ET-1 levels were increased in the patients (median 0.9 pg/ml; minimum 0.4 pg/ml; maximum 1.7 pg/ml) as compared with the control group (median 0.6 pg/ml; minimum under detection limit; maximum 0.8 pg/ml) (p = 0.04).

In addition, to verify whether the patient ET-1 values were related to the disease severity, a correlation test was performed on the patients' data. As shown in Fig. 1A, a significant correlation between serum ET-1 levels and PASI scores was observed (r = 0.60; p = 0.02).

**ET-1 in skin extracts**

Other experiments were undertaken to investigate whether the difference observed at the systemic level could also be revealed parametric tests were used: the Wilcoxon rank test, the Mann–Whitney test and, finally, the Spearman's rank correlation test.

**RESULTS**

**ET-1 in sera**

To compare serum ET-1 levels of psoriatic and normal subjects, 15 serum samples from the patients and 15 from the controls were assayed using ELISA techniques. The serum median ET-1 levels were increased in the patients (median 0.9 pg/ml; minimum 0.4 pg/ml; maximum 1.7 pg/ml) as compared with the control group (median 0.6 pg/ml; minimum under detection limit; maximum 0.8 pg/ml) (p = 0.04).

In addition, to verify whether the patient ET-1 values were related to the disease severity, a correlation test was performed on the patients' data. As shown in Fig. 1A, a significant correlation between serum ET-1 levels and PASI scores was observed (r = 0.60; p = 0.02).

**ET-1 in skin extracts**

Other experiments were undertaken to investigate whether the difference observed at the systemic level could also be revealed parametric tests were used: the Wilcoxon rank test, the Mann–Whitney test and, finally, the Spearman's rank correlation test.

**RESULTS**

**ET-1 in sera**

To compare serum ET-1 levels of psoriatic and normal subjects, 15 serum samples from the patients and 15 from the controls were assayed using ELISA techniques. The serum median ET-1 levels were increased in the patients (median 0.9 pg/ml; minimum 0.4 pg/ml; maximum 1.7 pg/ml) as compared with the control group (median 0.6 pg/ml; minimum under detection limit; maximum 0.8 pg/ml) (p = 0.04).

In addition, to verify whether the patient ET-1 values were related to the disease severity, a correlation test was performed on the patients' data. As shown in Fig. 1A, a significant correlation between serum ET-1 levels and PASI scores was observed (r = 0.60; p = 0.02).

**ET-1 in skin extracts**

Other experiments were undertaken to investigate whether the difference observed at the systemic level could also be revealed parametric tests were used: the Wilcoxon rank test, the Mann–Whitney test and, finally, the Spearman's rank correlation test.

**RESULTS**

**ET-1 in sera**

To compare serum ET-1 levels of psoriatic and normal subjects, 15 serum samples from the patients and 15 from the controls were assayed using ELISA techniques. The serum median ET-1 levels were increased in the patients (median 0.9 pg/ml; minimum 0.4 pg/ml; maximum 1.7 pg/ml) as compared with the control group (median 0.6 pg/ml; minimum under detection limit; maximum 0.8 pg/ml) (p = 0.04).

In addition, to verify whether the patient ET-1 values were related to the disease severity, a correlation test was performed on the patients' data. As shown in Fig. 1A, a significant correlation between serum ET-1 levels and PASI scores was observed (r = 0.60; p = 0.02).

**ET-1 in skin extracts**

Other experiments were undertaken to investigate whether the difference observed at the systemic level could also be revealed parametric tests were used: the Wilcoxon rank test, the Mann–Whitney test and, finally, the Spearman's rank correlation test.

**RESULTS**

**ET-1 in sera**

To compare serum ET-1 levels of psoriatic and normal subjects, 15 serum samples from the patients and 15 from the controls were assayed using ELISA techniques. The serum median ET-1 levels were increased in the patients (median 0.9 pg/ml; minimum 0.4 pg/ml; maximum 1.7 pg/ml) as compared with the control group (median 0.6 pg/ml; minimum under detection limit; maximum 0.8 pg/ml) (p = 0.04).

In addition, to verify whether the patient ET-1 values were related to the disease severity, a correlation test was performed on the patients' data. As shown in Fig. 1A, a significant correlation between serum ET-1 levels and PASI scores was observed (r = 0.60; p = 0.02).

**ET-1 in skin extracts**

Other experiments were undertaken to investigate whether the difference observed at the systemic level could also be revealed parametric tests were used: the Wilcoxon rank test, the Mann–Whitney test and, finally, the Spearman's rank correlation test.

**RESULTS**

**ET-1 in sera**

To compare serum ET-1 levels of psoriatic and normal subjects, 15 serum samples from the patients and 15 from the controls were assayed using ELISA techniques. The serum median ET-1 levels were increased in the patients (median 0.9 pg/ml; minimum 0.4 pg/ml; maximum 1.7 pg/ml) as compared with the control group (median 0.6 pg/ml; minimum under detection limit; maximum 0.8 pg/ml) (p = 0.04).

In addition, to verify whether the patient ET-1 values were related to the disease severity, a correlation test was performed on the patients' data. As shown in Fig. 1A, a significant correlation between serum ET-1 levels and PASI scores was observed (r = 0.60; p = 0.02).
Fig. 2. Distribution of the ET-1 and IL-8 levels evaluated in lesional (L), non-lesional (N) and control (CTR) skin extracts. Significances of comparisons are shown as the top. TP = total protein.

at the local level, comparing 8 lesional and 8 non-lesional skin extracts with 5 extracts from controls.

The analysis of the results showed a significant increase of ET-1 levels in lesional psoriatic skin (median 27.0 pg/mg TP, minimum 6.3 pg/mg TP; maximum 60.0 pg/mg TP) as compared with either non-lesional skin (median 14.5 pg/mg TP; minimum under detection limit; maximum 28.0 pg/mg TP; \( p = 0.01 \)) or normal skin (median 5.7 pg/mg TP, minimum under detection limit; maximum 23 pg/mg TP; \( p = 0.04 \)). No statistically significant difference was found between non-lesional and normal skin (\( p = 0.3 \)) (Fig. 2).

Furthermore, to explore the possibility that a quantitative relationship could exist between the lesional and the non-lesional skin of the patients, a correlation test between the corresponding ET-1 concentrations was performed: a significant regression coefficient was observed (\( r = 0.79, p = 0.03 \)).

As previously reported for serum ET-1 amounts, a correlation between lesional skin extract ET-1 levels and PASI scores was also noted (\( r = 0.80; p = 0.03 \)) (Fig. 1B).

No relationships were found in patients or controls, either at the systemic or the local level, between ET-1 concentrations and: age, sex, smoking history, and physical activity (data not shown).

### ET-1 mRNA in skin extracts

To verify and confirm results obtained by ET-1 ELISA from skin extracts, mRNA from small biopsies was extracted and specific messengers for ET-1 were reversely transcribed and amplified by RT-PCR. A single DNA fragment of the expected size (462 bp) was amplified in the skin samples. Fig. 3A shows the amplification signal of ET-1 mRNA from 2 psoriatic patients and one healthy volunteer. The specificity of this band was confirmed by southern blot analysis with a \(^{32}\)P-labelled internal probe (data not shown).

The densitometric analysis of the bands showed an increased expression of mRNA for ET-1 in lesional skin samples of psoriatic patients, compared with non lesional and with normal skin (Fig. 3B).

### IL-8 in skin extracts

IL-8 determinations are reported only for skin extracts, due to the fact that serum levels were mainly under detection limits.

The skin extract data (Fig. 2) confirmed that IL-8 is increased in lesional psoriatic skin (median 8.1 pg/µg TP; minimum 3.5 pg/µg TP; maximum 26.2 pg/µg TP) as compared to non-lesional (median 5 pg/µg TP; minimum 1.9 pg/µg TP; maximum 7.1 pg/µg TP; \( p = 0.02 \)) and normal skin (median 4.5 pg/µg TP; minimum 0.8 pg/µg TP; maximum 5.9 pg/µg TP; \( p = 0.04 \)). This is in agreement with previous findings published in the literature (13, 15).

Interestingly, at the lesional level, we could observe a significant correlation between ET-1 and IL-8 levels (\( r = 0.76, p = 0.05 \)) (Fig. 4). As expected, the above-mentioned results showed that IL-8 amounts were significantly correlated with the PASI scores (\( r = 0.78, p = 0.04 \)).
DISCUSSION

Previous reports have revealed a significant increase in ET-1 and ET-2 plasma levels in psoriatic patients, in comparison with the controls (19, 20).

Our study, showing increased serum ET-1 concentrations in psoriatic patients, also found a direct correlation between serum ET-1 levels and PASI scores, suggesting that ET-1 is linked to the PASI score, principally representing an index of the disease extension. Therefore, it is possible that serum ET-1 measurements can be useful in monitoring psoriasis.

To evaluate the possibility that the increased serum ET-1 concentrations could depend on lesional skin amounts, the present paper analyzed the ET-1 levels of lesional, non-lesional and normal skin. To standardize possible differences due to the biopsy variations, the data were expressed as pg/TP.

At the local level, we found that skin extracts of lesional psoriatic skin contained higher ET-1 concentrations as compared to non-lesional and normal skin, indicating that the highest levels of ET-1 were associated with the psoriatic plaque. The uninvolved skin did not show significant differences in comparison with the skin of normal individuals, although an evident correlation could be observed between ET-1 amounts measured in lesional and non-lesional skin of the same patients, suggesting a possible participation of the whole skin in the disease (21–23).

The data obtained at the protein level were confirmed at the mRNA level by RT-PCR analysis, performed in skin extracts. Thus, the same increasing concentration gradient of ET-1 is evident for normal, non-lesional and lesional skin.

As observed for serum ET-1 levels, skin extract ET-1 concentrations were also significantly correlated to the PASI scores, suggesting a direct relationship between the local ET-1 amounts and blood concentrations and between the latter and the disease severity score.

Since IL-8 is known to induce ET-1 synthesis in epithelial cells (24), and considering that IL-8 and ET-1 share several biological functions and that an involvement of IL-8 is already known in psoriasis, we measured the concentrations of this biological modulator in the same samples.

Interestingly, our results indicated that the lesional ET-1 behaviour paralleled that of IL-8. In fact, skin extract IL-8 was increased in the lesional skin and was significantly correlated to the PASI scores. No significant difference was found between normal and non-lesional skin. Finally, in accord with a possible ET-1 induction by IL-8, or alternatively a common induction by TNF-alpha (24), a significant correlation was observed between ET-1 and IL-8 amounts, at the lesional level.

Although this type of investigation does not provide information concerning the cellular source of this molecule, on the basis of the previous data, it is supposable that ET-1 mainly originates from endothelial cells, keratinocytes and mast cells.

Some speculations could be raised concerning the significance of the high ET-1 concentrations observed in involved areas of psoriatic skin.

In vitro studies clearly indicate that IL-8 induces ET-1 synthesis and that both act as mitogenic factors for the keratinocyte (6, 14, 24). In particular, the inhibition of the basal growth of keratinocytes in the presence of a specific antagonist for the ET receptors demonstrates that ET-1 is one of the most important factors involved in keratinocyte proliferation, which, in turn, could lead to abnormal proliferation and turnover of keratinocytes in psoriasis (6).

Since ET-1 is also mitogenic for endothelial and vascular smooth muscle cells (25), it could participate in the angiogenic processes (26), reported to be one of the earliest changes seen in the psoriatic lesion (27), probably driven by IL-8 and other cytokines, in the context of a more complex network.

A controversial point might be the discrepancy between the strong vasoconstrictive property of ET-1 and the marked dilatation of the papillary microvessels characteristic of psoriatic lesions and possibly due to the increased amounts of inducible nitric oxide synthase, recently reported (28). ET-1 increase may also be the result of endothelial cell activation (with subsequent ET-1 synthesis and release) and it is possible that ET-1, reflecting the activation of inflammatory processes, only represents the biological response directed to control vasoconstriction.

Interestingly, the mitogenic activity of this molecule both on keratinocyte and endothelium, together with its neoangiogenic properties, may contribute to maintain the psoriatic manifestations.

Finally, IL-8 and ET-1 molecules contribute to the leukocyte influx described in the lesional skin, due to their potent chemoattractant activities (8).

Although our findings are suggestive of a role of ET-1 in the psoriatic cytokine network, further investigations are in progress to better clarify its activity in the inflammatory phenomena associated with this dermatosis.

ACKNOWLEDGEMENTS

This work was supported in part by a grant from the Italian “Ministero della Sanita’’” “Psoriasis” and by AIRC.

REFERENCES


Acta Derm Venereol (Stockh) 78


