Acta Derm Venereol (Stockh) 1995; 75: 269–271

Comparison of the Effect of Hydrocortisone, Hydrocortisone-17-butyrate and Betamethasone on Collagen Synthesis in Human Skin *In vivo*

K.-M. HAAPASAARI[1], J. RISTELI[2], V. KOIVUKANGAS[1] and A. OIKARINEN[1]

Departments of [1]Dermatology and [2]Clinical Chemistry, University of Oulu, Oulu, Finland

It has been shown previously that topical corticosteroid treatment decreases collagen synthesis in human skin *in vivo* and that the adverse effects are due to reduced collagen synthesis. The aim of the present study was to evaluate the effect of hydrocortisone, hydrocortisone-17-butyrate and betamethasone on collagen synthesis in human skin *in vivo*.

Fourteen healthy male volunteers applied hydrocortisone, hydrocortisone-17-butyrate, betamethasone and vehicle twice a day for one week to four separate areas marked on their abdominal skin. The collagen synthesis rate in the skin was measured by assaying collagen propeptides from the suction blisters induced on the treated areas. Aminoterminal propeptide of type I procollagen (PINP) and aminoterminal propeptide of type III procollagen (PIIINP) were measured from skin blister fluid using radioimmunoassays. Skin thickness was measured with ultrasound.

Hydrocortisone decreased the two propeptides studied in the suction blister fluids less than did hydrocortisone-17-butyrate and betamethasone, but the interindividual variation was great. Hydrocortisone-17-butyrate and betamethasone had almost similar decreasing effects on the propeptides in the suction blister fluid. Hydrocortisone decreased the concentrations of PINP and PIIINP by about 35%. In some subjects (4/14) the decline of the collagen propeptide levels was over 50%. The decline in the concentration of PINP was 63% by hydrocortisone-17-butyrate and 69% by betamethasone, while the decrease in PIIINP was 55% by hydrocortisone-17-butyrate and 62% by betamethasone. None of the treatments had any effect on skin thickness within one week.

In conclusion, it seems that hydrocortisone is less atrophogenic than hydrocortisone-17-butyrate and betamethasone, as shown by radioimmunoassays for collagen propeptides. The order of inhibitory potency of the three glucocorticoids on collagen synthesis was hydrocortisone < hydrocortisone-17-butyrate < betamethasone. Thus, assay of collagen propeptides from suction blisters can be used to screen various steroids with respect to their action on collagen synthesis. *Key words: collagen propeptides; glucocorticoids.*

(Accepted January 4, 1995.)

Acta Derm Venereol (Stockh) 1995; 75: 269–271.

A. Oikarinen, Department of Dermatology, University of Oulu, FIN-90220, Finland.

Collagen comprises 70–80% of the dry weight of the dermis and is the most abundant protein in the skin. The two main collagen types in the skin are type I and type III collagen. The former is especially important, constituting 80–85% of all skin collagen, while the latter is a minor component making up 10–15% (1, 2). Collagens are synthesized as precursors. Aminoterminal and carboxyterminal propeptides are cleaved off from procollagen in the extracellular space, which enables the collagen molecules to assemble into functional fibers (3).

Previous methods for estimating collagen synthesis entail various problems, and most of these methods are unsuitable for clinical use (4). For this reason, a new method based on the radioimmunological assay of collagen propeptides has been developed. The availability of this assay has made it possible to measure the propeptides of type I and III collagen (5–7).

It has been demonstrated in previous studies that topical corticosteroid treatment reduces the levels of collagen I and III propeptides in skin blister fluid in human skin *in vivo*. The suction blister method has been shown to be highly sensitive for detecting changes in *de novo* collagen synthesis, even changes due to low potential steroids (8). To evaluate the atrophogenity of topical corticosteroids with different anti-inflammatory potencies, hydrocortisone (HC), hydrocortisone-17-butyrate (HB) and betamethasone (BM) were studied using the suction blister method, and aminoterminal propeptides of type I and III procollagen (PINP; PIIINP) were measured with radioimmunological assay.

MATERIAL AND METHODS

Fourteen healthy male volunteers (mean age 26, range 21–44) participated in the trial. On their abdominal skin, four areas were marked (size 10 cm × 10 cm). The areas were treated for one week twice a day with four different ointments: HC (10 mg/g), HB (1 mg/g), BM (1 mg/g) and vehicle, which served as a control. Neither the investigator nor the subjects were aware of the quality of the ointments applied to these four areas. After one week of treatment, skin thickness was measured with ultrasound and suction blisters were induced on these four areas as described previously (9). The blister fluids were collected and kept frozen until the radioimmunological assays, in which PINP and PIIINP were measured (6, 7). The person analysing the propeptide levels did not know which of the four areas the samples were from.

The proportion of the synthesis of type I and III procollagens was calculated on the basis of molecular masses of procollagen propeptides. The molecular mass of PINP is 35,000 and that of PIIINP is 42,000. For the statistical analysis, a logarithmic transform of the original values (procollagen propeptide concentrations) was taken to obtain a more symmetric and Gaussian-like distribution. The analysis was performed using paired-samples *t*-test.

The protocol had been approved by the ethical committee of the Medical Faculty of the University of Oulu, Finland.

RESULTS

Effects of different glucocorticoids on PINP and PIIINP in suction blister fluid

The concentrations of PINP and PIIINP were affected by glucocorticoid treatment. For the statistical analysis, a logarithmic transformation of the original values was taken because of the skewness of the distribution of the propeptide concentrations.

 ISSN 0001-5555

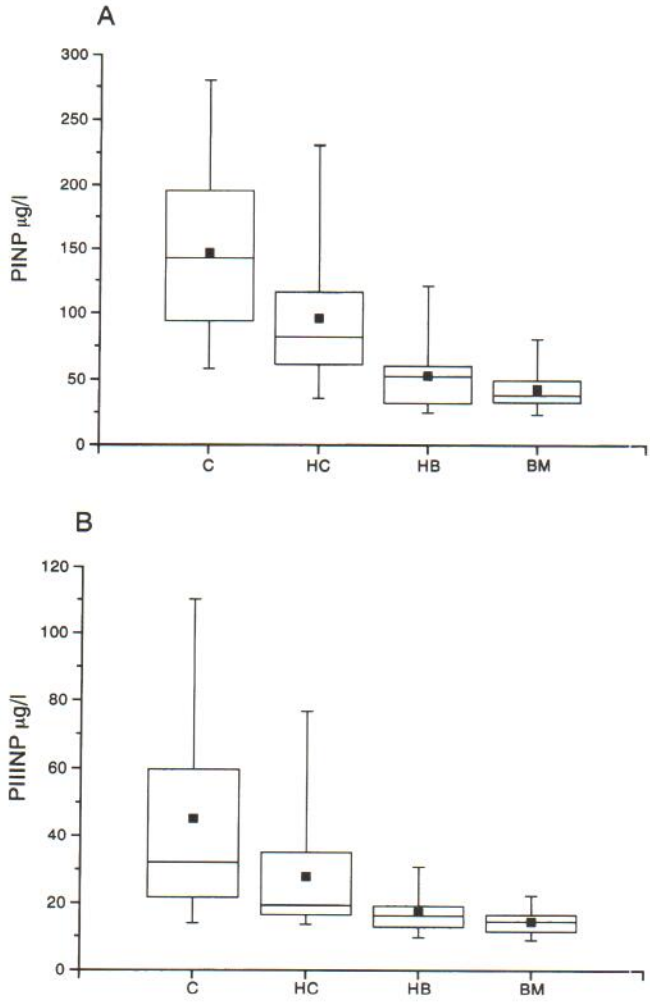

*Fig. 1*A,B. The concentrations of PINP and PIIINP in suction blister fluids treated by hydrocortisone (*HC*), hydrocortisone-17-butyrate (*HB*), betamethasone (*BM*) and vehicle (*C*). The bottom of the box marks the 25th percentile and the top of the box the 75th percentile (the limits within which 50% of the values fall). The square symbol in the box marks the mean and the vertical line median. The vertical lines represent the ranges.

PINP values. The geometric mean of the ratios of the concentrations between HC and vehicle (C) treatments was 0.65 (95% confidence interval 0.47, 0.89; $p<0.01$), between HB and C 0.37 (95% CI 0.27, 0.50; $p<0.001$), between BM and C 0.31 (95% CI 0.24, 0.42; $p<0.001$) and between BM and HB 0.85 (95% CI 0.70, 1.01; $p<0.07$) (Fig. 1A).

PIIINP values. The geometric mean of the ratios of the concentrations between HC and C treatments was 0.66 (95% CI 0.47, 0.91; $p<0.01$), between HB and C 0.45 (95% CI 0.31, 0.65; $p<0.001$), between BM and C 0.38 (95% CI 0.27, 0.55; $p<0.001$) and between BM and HB 0.85 (95% CI 0.70, 1.04; $p<0.11$) (Fig. 1B).

HC decreased the concentrations of PINP and PIIINP by about 35%. In some subjects (4/14) the decline of the collagen propeptide levels was over 50%. The decline in the concentration of PINP was 63% by HB and 69% by BM, while the decrease in PIIINP was 55% by HB and 62% by BM.

The decrease of PIIINP correlated significantly with the decrease of PINP with the tested glucocorticoids (see Fig. 2 as an example).

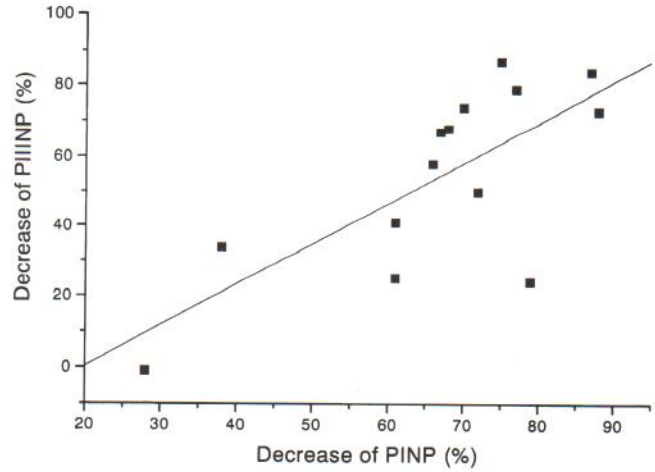

Fig. 2. The correlation between the decreases in PIIINP and PINP when the skin was treated with betamethasone ($r=0.728$, $p=0.003$).

The decrease of the propeptides by one glucocorticoid correlated significantly with the decrease induced by another glucocorticoid (see Fig. 3).

The proportion of the type III procollagen to the total type I and III procollagens was calculated on the basis of the molecular masses of the procollagen propeptides. The mean proportion of type III collagen was 20% on the area treated by vehicle (standard deviation 0.063), 20% by HC (SD 0.053), 23% by HB (SD 0.059) and 23% by BM (SD 0.056).

The mean of skin thickness was 1.72 mm (SD 0.19) in the HC area, 1.77 mm (SD 0.23) in the HB area, 1.65 mm (SD 0.22) in the BM area, and 1.75 mm (SD 0.23) in the vehicle-treated area.

DISCUSSION

All the tested topical glucocorticoids decreased collagen propeptide concentrations in the suction blister fluids. Even HC, which is generally considered to be relatively harmless in respect to skin atrophy, caused significant decrease in propeptides,

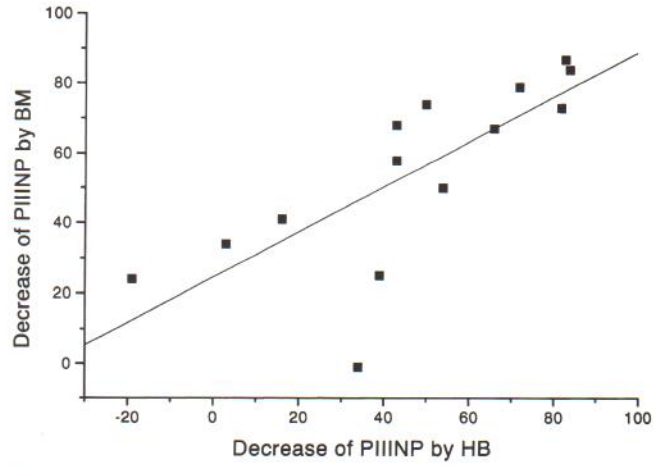

Fig. 3. The correlation between the decrease of PIIINP by hydrocortisone and by betamethasone ($r=0.752$, $p=0.0019$).

suggesting that it may also cause skin atrophy in suspectible subjects. This finding is also in line with numerous animal and cell culture studies, in which HC in clinically relevant doses has been shown to decrease collagen synthesis and the corresponding messenger RNA levels (10–12). In addition, in humans HC and HB have been demonstrated to cause skin atrophy when skin thickness was estimated by radiological methods (13) and to decrease activity of prolyl hydroxylase, a key enzyme in collagen synthesis (14).

Even though BM and HB cause a 60–70% decrease in the concentration of PINP and PIIINP, neither of these caused any significant reduction in skin thickness within one week, or clinically visible atrophy. The reason for this is the long half-life of collagen in the skin (15), which means that only a relatively long (several months) continuous treatment period would induce clinical atrophy.

In conclusion, we have shown that a newly developed *in vivo* assay can be used to screen various steroids with respect to their action on collagen synthesis. This method seems to be especially useful for screening different pharmacological compounds affecting the skin and for minimizing the adverse effects of topical steroids. By combining clinical evaluation of skin atrophy and the measurement of the actual collagen synthesis rate *in vivo*, our method can give useful information about the real atrophy risk of old and new corticosteroids.

ACKNOWLEDGMENTS

We acknowledge the expert technical assistance of Ms. Kristiina Pekkala and Ms. Eeva-Liisa Kokkonen and thank Esa Läärä, M.Sc., for advice in statistical questions. This study was supported by the Medical Research Council of the Academy of Finland.

REFERENCES

1. Bauer EA, Uitto J. Collagen in cutaneous diseases. Int J Dermatol 1979; 8: 251–271.
2. Bornstein P, Traub W. The chemistry and biology of collagen. In: Neurath H, Hill RL, eds. The proteins. Vol 4. New York: Academic Press, 1979: 411–632.
3. Procop DJ, Kivirikko KI, Tudermann L, Guzman NA. The biosynthesis of collagen and its disorders. N Engl J Med 1979; 301: 12–23, 77–85.
4. Annala A-P, Risteli L, Koivukangas V, Autio P, Oikarinen A. Measurement of collagen metabolism in skin diseases. A review of old and new techniques and their clinical applications. Eur J Dermatol 1993; 3: 696–703.
5. Melkko J, Niemi S, Risteli L, Risteli J. Radioimmunoassay of the carboxyterminal propeptide of human type I procollagen. Clin Chem 1990; 7: 1328–1332.
6. Risteli L, Risteli J. Noninvasive methods for detection of organ fibrosis. In: Rojkind M, ed. Connective tissue in health and in disease. Boca Raton: CRC Press, 1992: 61–98.
7. Risteli L, Niemi S, Trivedi P, Mäentausta O, Mowat AP, Risteli L. Rapid equilibrium radioimmunoassay for the aminoterminal propeptide of human type III procollagen. Clin Chem 1988; 34: 715–718.
8. Oikarinen A, Autio P, Kiistala U, Risteli L, Risteli J. A new method to measure type I and III collagen synthesis in human skin in vivo: demonstration of decreased collagen synthesis after topical glucocorticoid treatment. J Invest Dermatol 1992; 98: 220–225.
9. Kiistala U. Suction blister device for separation of viable epidermis from dermis. J Invest Dermatol 1968; 50: 129–137.
10. Cutroneo KR, Rokowski R, Counts DF. Glucocorticoids and collagen synthesis: comparison in in vivo and cell culture studies. Coll Relat Res 1981; 1: 557–568.
11. Oikarinen A, Uitto J, Oikarinen J. Glucocorticoid action on connective tissue: from molecular mechanisms to clinical practise. Med Biol 1986; 64: 221–230.
12. Oikarinen J, Pihlajaniemi T, Hämäläinen L, Kivirikko KI. Cortisol decreases the cellular concentration of translatable procollagen mRNA species in cultured human skin fibroblasts. Biochim Biophys Acta 1983; 741: 297–302.
13. Marks R. Methods for the assessment of skin atrophogenity of topical corticosteroids. Dermatol 1976; Suppl 152: 117–126.
14. Oikarinen A, Hannuksela M. Effect of hydrocortisone-17-butyrate, hydrocortisone and clobetasol-17-propionate on prolyl hydroxylase activity in human skin. Arch Dermatol Res 1980; 267: 79–82.
15. Kivirikko KI. Urinary excretion of hydroxyproline in health and in disease. Int Rev Connect Tissue Res 1970; 5: 93–163.