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Effects of long-term inhaled corticosteroids on skin collagen synthesis and thickness in asthmatic patients

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ABSTRACT: There are only a few studies on the adverse effects of inhaled corticosteroids on the skin in asthmatic patients. Therefore, we evaluated the effect of inhaled corticosteroids on *de novo* collagen synthesis of skin and bone, skin thickness and the total amount of skin collagen.

Twenty seven consecutive new asthmatic patients, on a moderate dose of budesonide or beclomethasone dipropionate, were invited to take part in this prospective study. Radioimmunological analyses of aminoterminal propeptides of type I and III procollagens (PINP, PIIINP, respectively) in suction blister fluid (SBF) of skin and in serum and carboxyterminal propeptide of type I procollagen (PICP) and cross-linked carboxyterminal telopeptide of type I collagen (ICTP) in serum were performed at entry and after 3 and 6 months of inhaled corticosteroid treatment. Ultrasound measurements of skin thickness at two sites were performed at entry, at 3 and 6 months and after 1–2 yrs of inhaled corticosteroid treatment in 20 patients, six of whom had been prescribed one or more courses of oral corticosteroids. Skin hydroxyproline of punch biopsies was determined to measure the total amount of skin collagen (males, at entry and at 6 months).

Skin thickness and the total amount of skin collagen on the abdomen were unchanged after 1–2 yrs of inhaled corticosteroid use. A slight decrease was observed in the upper arm skin thickness, especially in those subjects who had received inhaled plus oral corticosteroids. The procollagen propeptide concentrations (PINP, PIIINP) were markedly decreased in SBF at 3 months and remained at this level at 6 months. In serum, a slight decrease was seen in the PINP, PIIINP and ICTP concentrations at 3 and 6 months.

In conclusion, inhaled corticosteroids decrease the collagen synthesis of skin and bone, but skin thickness and the total amount of collagen in skin are not changed markedly after 1–2 yrs of treatment.

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Inhaled corticosteroids (ICs) have been recommended as first-line therapy for all but the mildest forms of asthma. The treatment is likely to be long and the question of safety is important. The adverse effects associated with this treatment are well known, such as thrush, dysphonia, sore throat, changes in lipid and bone metabolism, growth retardation and cataract [1]. Furthermore, ICs also have an effect on skin. Six week therapy with ICs is sufficient to repress collagen synthesis of skin [2]. Purpura and dermal thinning have been associated with high-dose IC treatment [3].

Collagen is the most abundant protein in humans. Type I collagen comprises 90% of the bone organic matrix, and in skin it is the major structural component of dermis, most of which consists of type I collagen (80%), the rest being mostly type III collagen (10–15%) [4].

Collagen is synthesized in a precursor form, procollagen. During synthesis, procollagen propeptides are cleaved off by specific proteases enabling collagen molecules to assemble into functional fibres [5]. Thus, the amount of these propeptides reflects *de novo* synthesis of collagen.

Radioimmunological analysis of procollagen propeptides in serum [6–8] and extracellular fluid [9] is a new clinical tool for monitoring the collagen synthesis rate in various clinical conditions, including the effect of topical and systemic corticosteroid treatments [9, 10]. We have shown recently that the decrease of collagen propeptides in skin suction blister fluid (SBF) is associated with a decline of type I collagen messenger ribonucleic amino acid (RNA) levels (unpublished data).

There have been no studies assessing simultaneously both the synthesis and the net amount of collagen in skin in asthma patients receiving ICs. We therefore studied the effect of IC treatment on collagen synthesis and the amount of collagen in skin and the collagen synthesis and degradation in bone in asthma patients. A special emphasis was placed on the evaluation of the risk of developing clinical skin atrophy in patients treated with ICs.

Materials and methods

Subjects

Twenty seven newly diagnosed asthmatics (using the criteria of the American Thoracic Society [11]) were invited to take part in this prospective study. For the treatment of asthma, a moderate dose (800–1000 μg) of inhaled corticosteroid (budesonide, BUD, or beclomethasone dipropionate, BDP) was prescribed. Two patients received a higher dose of BUD (1200 μg and 1600 μg, respectively). Twenty three patients were treated with BUD. Nineteen of them used Pulmicort® Turbuhaler® (Astra, Espoo, Finland) and four used Cortivent® (Leiras, Turku, Finland) inhalation aerosol with a spacer, Rondo® (Leiras). Three patients treated with BDP (Becotide®, Glaxo Wellcome, Espoo, Finland) had a spacer, Volumatic® (Glaxo Wellcome), and one patient was treated with inhalation powder (Becotide Rotadisk®, Glaxo Wellcome). All patients also used an inhaled β_2-agonist as needed. None had previously been on inhaled or topical corticosteroids, or had diseases affecting collagen metabolism. Table 1 shows the initial data.

A written informed consent was obtained from all the patients. This study was approved by the Ethics Committee of the Faculty of Medicine and the Oulu University Hospital.

Study design

Skin thickness was measured with ultrasound (Dermascan A, Cortex technology, Hadsund, Denmark) on the abdomen and the upper right arm before the treatment and at 3 and at 6 months. Three individual measurements were made, and the mean was calculated at each site. At these time-points, suction blisters were induced on the abdominal skin using a disposable suction blister device (Dermouac®, Ventipress, Lappeenranta, Finland) for the majority of patients during the follow-up and a plastic suction blister device for some patients at entry [12]. Blister fluid was collected and kept frozen until the radioimmunological assays of aminoterminal propeptides of type I and III procollagens (PINP, PIIINP, respectively) [6, 8]. Serum samples were obtained at the baseline and at 3 and 6 months. From these samples, carboxy- and aminoterminal propeptides of type I procollagen (PICP, PINP, respectively) [6, 7], PIIINP [8] and cross-linked carboxyterminal telopeptide of type I procollagen (ICTP) [13] were analysed using radioimmunological assays.

Table 1. – Patients and the initial treatment

	Patients n
Sex male/female	19/8
Age+ yrs	38 (16–68)
Smoking	
Nonsmoker	7
Former	16
Current	4
Inhaled corticosteroids	
BUD 800 μg	22
BUD 1600 μg	1
BDP 800 μg	1
BDP 1000 μg	3

+: mean (range).

Skin punch biopsies (diameter 4 mm) were taken from the abdominal skin of 20 male subjects at the baseline and at 6 months. From these biopsies, skin hydroxyproline was determined using a colorimetric method [14].

After 1–2 yrs of IC use, the subjects were invited to attend for a further measurement of skin thickness. Twenty of them consented, and skin thickness was measured on the upper arm and the abdominal skin by ultrasound. Six of these patients had received one or more courses of oral corticosteroids after the initial 6 month follow-up.

As controls, skin thickness of upper arm, serum PINP, PIIINP and ICTP levels and PINP and PIIINP values of blister fluid of 14 healthy females (median age 51 yrs, range 47–54 yrs) were followed for 6 months using the same method. PINP and PIIINP in SBF were also followed in eight male volunteers for 1 yr (median age 24 yrs, range 22–32 yrs).

Statistical analysis

A logarithmic transformation of the original values (procollagen propeptide concentrations) was taken to obtain a more Gaussian-like distribution. For the statistical analysis, paired-sample t-test Statistical Products and Service Solutions (SPSS) for Windows was used to compare the values obtained at 3 and 6 months to those obtained at entry.

Results

Skin thickness and skin hydroxyproline

Considering all 27 subjects, abdominal skin thickness did not change during the 6 month follow-up (table 2). On the upper arm, a slight decrease was seen (mean 0.09 mm, 95% confidence interval (CI) 0.04–0.14 mm, p=0.001) at 6 months (females plus males). In females, the decrease was 0.05 mm (95% CI 0.003–0.105 mm, p=0.041) and in males 0.1 mm (95% CI 0.031–0.167 mm, p=0.007).

One to two years after the beginning of the study, the skin thickness of 20 patients was measured on the abdomen and the upper arm. Fourteen patients had used IC treatment only. The skin thickness of the abdomen and the

Table 2. – The skin thickness on the upper arm and abdomen, aminoterminal propeptides of type I (PINP) and III (PIIINP) procollagens in suction blister fluid of skin and skin hydroxyproline

	Time after treatment months		
	0	3	6
Skin thickness mm			
Upper arm	1.64 (1.14–1.95)	1.56 (1.15–1.95)	1.50 (1.11–1.87)
Abdomen	2.01 (1.16–2.54)	2.00 (1.16–2.52)	2.00 (1.12–2.57)
PINP $\mu g \cdot L^{-1}$	557 (70–4580)	134 (40–1158)	167 (28–1045)
PIIINP $\mu g \cdot L^{-1}$	244 (16–1580)	38 (12–1040)	60 (7–620)
Skin hydroxyproline $\mu g \cdot mm^{-2}$	10 (6–23)	ND	12 (7–15)

Values are expressed as median (range in parenthesis). The data from female and male patients are combined. ND: not determined.

upper arm was unchanged compared to the measurement at entry or at 6 months. The mean (range) abdominal skin thickness of these 14 patients was 2.06 mm (1.75–2.54 mm) at entry, 2.06 mm (1.75–2.55 mm) at 6 months and 2.09 mm (1.74–2.91 mm) after 1–2 yrs. The mean (range) upper arm skin thickness was 1.56 mm (1.22–1.95 mm) at entry, 1.50 mm (1.14–1.86 mm) at 6 months and 1.55 mm (1.06–2.08 mm) after 1–2 yrs.

Six patients, who had received IC plus oral corticosteroid treatment for one to several weeks after the 6 month follow-up, showed thinning of the skin on the upper arm (table 3). No such change was observed in abdominal skin thickness.

Table 3 shows the individual upper arm skin thickness values and PINP concentrations before the beginning of IC treatment and during the follow-up in male and female patients separately. The initial values of upper arm skin thickness and PINP in SBF were lower in females than in males. The ratio of upper arm skin thickness in females *versus* males was 0.82. In control females, median (range) skin thickness of the upper arm was 1.43 mm (1.05–2.00 mm) at entry and 1.37 mm (1.03–2.05 mm) at 6 months. These values are close to those found in IC-treated females (see table 3).

The total amount of skin collagen, expressed as skin hydroxyproline $\mu g \cdot mm^{-2}$, did not change during the 6 month follow-up (table 2).

Collagen propeptides in SBF in males and females

PINP had decreased by 66% (95% CI 51–77%, $p< 0.001$) at 3 months and by 70% (95% CI 54–80%, $p< 0.001$) at 6 months. PIIINP had decreased by 76% (95% CI 60–86%, $p<0.001$) at 3 months and by 76% (95% CI 62–85%, $p<0.001$) at 6 months. The medians and ranges of the propeptide concentrations in SBF are shown summarized in table 2. The individual values for PINP for males and females separately are shown in table 3. In female controls, the median (range) of PINP levels in SBF was 175 $\mu g \cdot L^{-1}$ (48–362 $\mu g \cdot L^{-1}$) at entry and 273 $\mu g \cdot L^{-1}$ (59–593 $\mu g \cdot L^{-1}$) after 6 months. The corresponding PIIINP levels were 58 $\mu g \cdot L^{-1}$ (20–208 $\mu g \cdot L^{-1}$) at entry and 127 $\mu g \cdot L^{-1}$ (14–213 $\mu g \cdot L^{-1}$) after 6 months.

In male controls, the median (range) of PINP in SBF was 216 $\mu g \cdot L^{-1}$ (136–705 $\mu g \cdot L^{-1}$) at entry and 236 $\mu g \cdot L^{-1}$ (87–844 $\mu g \cdot L^{-1}$) after 1 yr. The median (range) of PIIINP was 58 $\mu g \cdot L^{-1}$ (28–168 $\mu g \cdot L^{-1}$) of entry and 57 $\mu g \cdot L^{-1}$ (16–196 $\mu g \cdot L^{-1}$) after 1 yr.

Table 3. – Individual data on upper arm skin thickness and PINP values in suction blister fluid before IC treatment (0 months) and after 3 months, 6 months and 1–2 yrs

Subject No.	Age yrs	Skin thickness mm				PINP in SBF $\mu g \cdot L^{-1}$			Dose of IC µg
		0 months	3 months	6 months	1–2 yrs	0 months	3 months	6 months	
Males									
1	16	1.46	1.41	ND	ND	500	628	ND	800 BUD
2	17	1.95	1.89	1.85	1.96	1018	328	308	800 BUD
3	18	1.39	1.40	1.28	1.54	4580	1158	200	800 BUD
4	23	1.83	1.82	1.69	2.08	358	380	110	800 BUD
5	24	1.83	1.59	1.58	ND	793	110	168	800 BUD
6	27	1.80	1.78	1.76	1.55*	695	338	440	800 BUD
7	27	1.36	1.35	1.34	1.37	615	125	188	800 BUD
8	33	1.87	1.90	1.86	1.85	1138	90	125	800 BUD
9	36	1.81	1.78	1.49	ND	2360	593	1045	1000 BUD
10	37	1.47	1.47	1.59	ND	333	40	295	800 BUD
11	40	1.71	1.71	1.67	1.65*	1208	143	795	1600 BUD
12	42	1.37	1.43	1.41	1.36	733	255	165	800 BUD
13	42	1.71	1.59	1.50	1.62	803	135	288	800 BUD
14	48	1.93	1.95	1.73	1.74	1120	108	78	800 BUD
15	50	1.56	1.69	1.61	1.60	870	455	270	800 BUD
16	50	1.76	1.74	1.70	1.65*	425	75	28	800 BDP
17	50	1.90	1.91	1.87	ND	70	500	315	800 BUD
18	64	1.62	1.70	ND	ND	340	83	ND	800 BUD
19	68	1.73	1.37	1.37	ND	498	133	155	800 BUD
Median	37	1.73	1.70	1.67	1.64	733	143	200	
Females									
1	17	1.22	1.20	1.14	1.32	205	178	63	800 BUD
2	32	1.41	1.25	1.38	1.16	230	108	40	800 BUD
3	35	1.49	1.53	1.46	1.51	323	138	70	800 BUD
4	37	1.23	1.24	1.26	1.06	113	78	183	800 BUD
5	41	1.57	1.36	1.46	1.49	273	95	110	1000 BDP
6	44	1.47	1.34	1.34	1.24*	325	118	80	800 BUD
7	49	1.41	1.37	1.41	1.16*	265	55	ND	800 BUD
8	66	1.14	1.15	1.11	1.02*	208	110	55	1000 BDP
Median	39	1.41	1.30	1.36	1.20	237	109	95	

*: The patient received one or more courses of oral corticosteroids after a 6 month follow-up. ND: not determined. BUD: budesonide; BDP: beclomethasone dipropionate; IC: inhaled corticosteroid; SBF: suction blister fluid; PINP: aminoterminal propeptide of type I procollagen.

Collagen synthesis and degradation markers in serum

PINP had decreased by 13% (95% CI 1–23%, p=0.033) and ICTP by 12% (95% CI 0–23%, p=0.057) at 3 months. PINP showed an average decrease of 13% (95% CI ranged from a 29% decrease to a 6% increase) at 6 months, but this was not statistically significant (p=0.16). ICTP behaved similarly. The mean decrease of ICTP was 12% (95% CI ranged from a 26% decrease to a 4% increase) at 6 months, and this decrease was also not statistically significant (p=0.13) (fig. 1).

The PICP concentration in serum was unchanged during the 6 months (data not shown).

PIIINP had decreased by 22% (95% CI 11–32%, p= 0.001) at 3 months and by 20% (95% CI 4–34%, p=0.023) at 6 months, and no further decrease was hence observed.

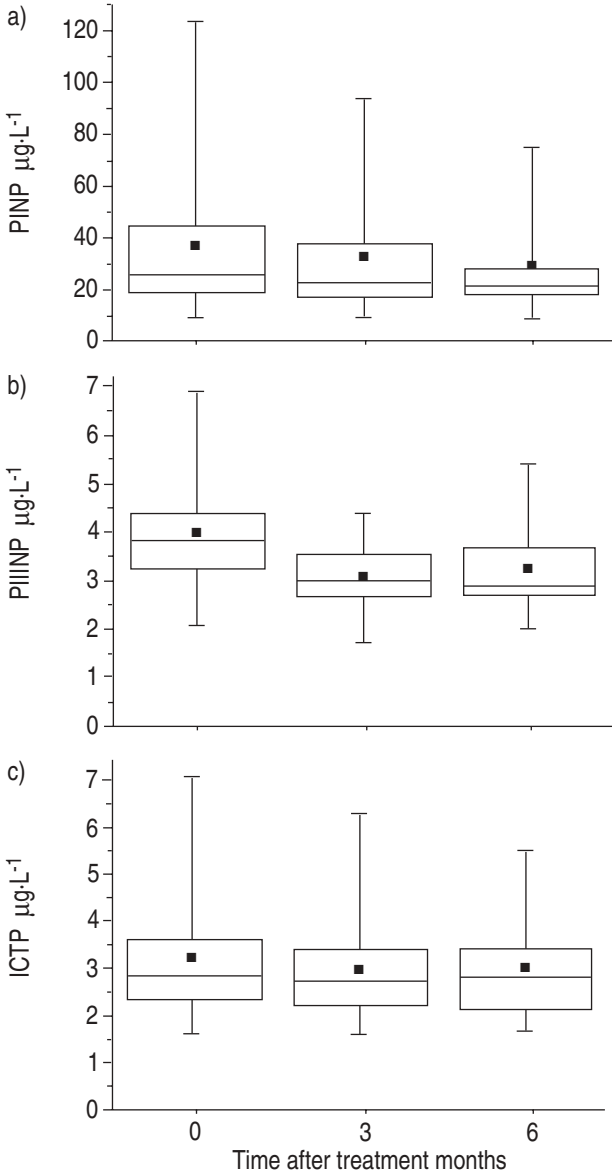

Fig. 1. – Concentrations of a) PINP; b) PIIINP; and c) ICTP in serum samples. A slight decrease of PINP and ICTP was observed at 3 months, but at 6 months the downward tendency was not so obvious. PIIINP was decreased at both 3 and 6 months. ■ : mean. Horizontal lines represent the median, boxes represent the interquartile range and whiskers represent the range. PINP, PIIINP: aminoterminal propeptides of type I and III procollagens, respectively; ICTP: cross-linked carboxyterminal telopeptide of type I collagen.

The procollagen propeptides, PINP, PIIINP, PICP and ICTP, were analysed in serum of female controls. During the 6 month follow-up, the level of the propeptides was unchanged. The median (range) level of PINP in serum was 44.4 µg·L^{-1} (18–101 µg·L^{-1}) at entry and 46.7 µg·L^{-1} (18.5–103.9 µg·L^{-1}) after 6 months. The median (range) level of PIIINP was 3.5 µg·L^{-1} (2.2–11.5 µg·L^{-1}) at entry and 3.5 µg·L^{-1} (3–11.6 µg·L^{-1}) after 6 months. The median (range) level of PICP was 107.7 µg·L^{-1} (74.5–191.8 µg·L^{-1}) at entry and 114.1 µg·L^{-1} (63.4–198.5 µg·L^{-1}) after 6 months. The median (range) level of ICTP was 2.7 µg·L^{-1} (1.7–5.4 µg·L^{-1}) at entry and 2.9 µg·L^{-1} (1.9–4.7 µg·L^{-1}) after 6 months. There are no data of PINP, PIIINP and ICTP of male controls.

Discussion

We demonstrated here that the moderate IC doses generally used for the treatment of asthma are able to decrease skin and bone collagen synthesis. As the measurement of skin thickness and the total amount of skin collagen shows, use of ICs alone does not lead to marked thinning of the skin.

The study was planned to take place in a real clinical situation, and the patients were recruited into the study on the basis of the diagnosis of asthma and the initiation of treatment with ICs. In Finland, all adult asthma patients are treated with continuous ICs as a first-line treatment (except the mildest forms of asthma), and thus no untreated asthma control group was available.

We have previously measured the collagen propeptide levels reflecting collagen synthesis *in vivo* in patients aged 20–80 yrs with the method used in the present study, and the levels were of the same order as the pretreatment values of the present subjects [15]. In contrast, the levels noted during the IC treatment were clearly lower than the corresponding control levels recorded with the same method earlier [15]. In addition, we have previously shown that short-term treatment of young asthmatics with nedocromil does not decrease the levels of skin collagen synthesis [2]. Furthermore, we followed up the collagen synthesis of 14 healthy female volunteers for 6 months and eight male volunteers for 12 months and saw no decreases in propeptide levels. We are hence confident that collagen synthesis is decreased by ICs.

Female skin thickness seems to be smaller than male skin thickness. In the present study, the upper arm skin was about 20% thinner in females than in males, a finding in agreement with the previous studies [16]. There are data to suggest that females are more likely to develop skin bruising [17]. This finding was partially supported by our study, especially the results obtained after 1–2 yrs of treatment with IC plus oral corticosteroid, since the skin thickness of the upper arm had decreased from a median of 1.41 to 1.20 mm. The corresponding decrease in males was from 1.73 to 1.67 mm.

Since older patients seem to get skin bruising more often than younger patients [17], it would be interesting to compare young and old asthma patients separately. Unfortunately, most of the present asthma patients studied were middle-aged, and the data are hence not sufficient for statistical analysis.

CAPEWELL *et al*. [3] demonstrated that high-dose use of ICs for 4 yrs is associated with purpura and dermal thinning. However, a confounding influence may have been caused by previous courses of oral prednisolone. No such changes were seen in the patients who had received low-dose ICs [3]. Thus, if the IC treatment is combined with prednisolone perorally, the increased dose probably leads to skin thinning, as it did in these patients. However, the limited number of patients did not permit us to draw firm conclusions.

In previous studies, PICP has been used as a marker of bone collagen synthesis [7] and ICTP as a marker of bone degradation [12]. The previous results on the effect of ICs on bone collagen metabolism have been quite contradictory. It has been postulated that ICs may have a short-term effect on bone metabolism, but the changes are balanced in long-term use [18, 19]. Bone resorption has been studied as well, and the results are contradictory. However, osteocalcin has been used in several studies, but this parameter does not accurately reflect bone formation [19]. In this study, we used serum procollagen propeptides as a marker of *de novo* synthesis of collagen, and found that they were decreased in patients on IC therapy. The degradation marker of type I collagen (ICTP) in serum decreased almost to the same extent as the synthesis marker PINP in serum, suggesting that the degradation of bone collagen may also be decreased by ICs *in vivo*.

Unfortunately, we are not able to measure type I collagen degradation in SBF, but the unchanged skin thickness confirms that the decrease of synthesis does not lead to a thinning of the skin after the use of moderate doses of ICs. This is possibly due to the fact that the degradation and turnover of collagen slow down, so that the net amount of collagen remains relatively constant. This assumption is supported by numerous cell culture studies, which have shown that corticosteroids decrease the activities of matrix metalloproteinases, *i.e*. enzymes that degrade collagens [20]. This could also explain partially why even a marked decrease in the *de novo* synthesis of collagen in soft tissues, including skin, did not result in a significant change in the amount of collagen.

The aim of this study was to evaluate the effects of ICs alone on skin and bone. Skin thickness was therefore measured after 1–2 yrs in all of the present patients who consented and their treatments were carefully analysed. Six patients had used oral corticosteroids after the initial 6 month study period. We found skin thickness to be decreased in the asthmatics who had received both ICs and oral corticosteroids on a periodical basis. There thus seems to be a patient group receiving both oral corticosteroids and ICs at enhanced risk for skin atrophy. This finding is in accordance with the study of CAPEWELL *et al.* [3].

In conclusion, patients receiving only moderate doses of inhaled corticosteroids need not generally be monitored for skin atrophy. However, possible skin atrophy should be monitored in certain high-risk individuals, *i.e*. patients receiving inhaled plus oral corticosteroids periodically, and at certain body sites such as arms.

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