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Stimulation of collagen biosynthesis by topically applied vitamin C

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Introduction

Vitamin C, the antiscorbutic (ascorbic acid) factor plays a dual role in life. It is a required cofactor for several hydroxylases and monooxidases. It is also a most significant scavenger of free radicals by allowing vitamin E to remain in its active form.

In the dermis, vitamin C is required for the formation of hydroxyprolyl residues to form stable triple-helical collagen molecules, and hydroxylysyl residues operating in crosslinks synthesis. In fibroblasts culture, vitamin C is also known to stabilize the collagen messenger RNAs (m-RNA) and increase procollagen synthesis. The activity in the dermis of vitamin C topically applied on normal human skin is demonstrated in the present study by observing an increased steady-state level of the m-RNA for the two major collagen molecules (I and III) in skin, three of their postradiational processing enzymes (N- and C-procollagen peptidases and lysyloxidase) and markers of dermal and epidermal cells activity.

Material

- Ten female volunteers 50 to 60 years-old;
- application of w/o emulsion containing 5% L-ascorbic acid* on the external face of one arm and the w/o emulsion (Placebo) on the external face of the other arm, double blind, daily for 6 months;
- 5 mm punch biopsy at the location of the topical treatment (approval ethical committee);
- conservation in liquid N2:
- pulverisation in "Microdismembrator" - Braun;
- solubilisation in 5M guanidium isothiocyanate, 0.1 M β-mercaptoethanol;
- collection of ARN by ultracentrifugation on a cesium chloride cushion (average 3 µg total RNA per biopsy).

Methods: measurement of m- and r-RNAs

- Ten ng of total RNA;
- RT-PCR amplification (20 to 35 cycles) using specific primers simultaneously with a defined number of copies of a synthetic RNA specific of each m-RNA using the same primers but generating an amplification product of a different size;
- densitometric measurement of the reaction products after acrylamide gel electrophoresis.

Definition of the sample

- 28S ribosomal RNA (r-RNA);
- Vimentin (dermis) (Vim);
- Keratin 10 (epidermis) (K10).

Specific m-RNAs

- α1 I collagen (α1 I);
- α1 III collagen (α1 III);
- procollagen N and C protease (N-PCP, C-PCP) (processing enzymes);
- lysyloxidase (LO) (crosslinking enzyme);
- matrix metalloproteinases 1, 2 and 9 (MMP1, MMP2 and MMP9).

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Expression of results

In units or number of copies per unit of r-RNA.

Statistical analysis

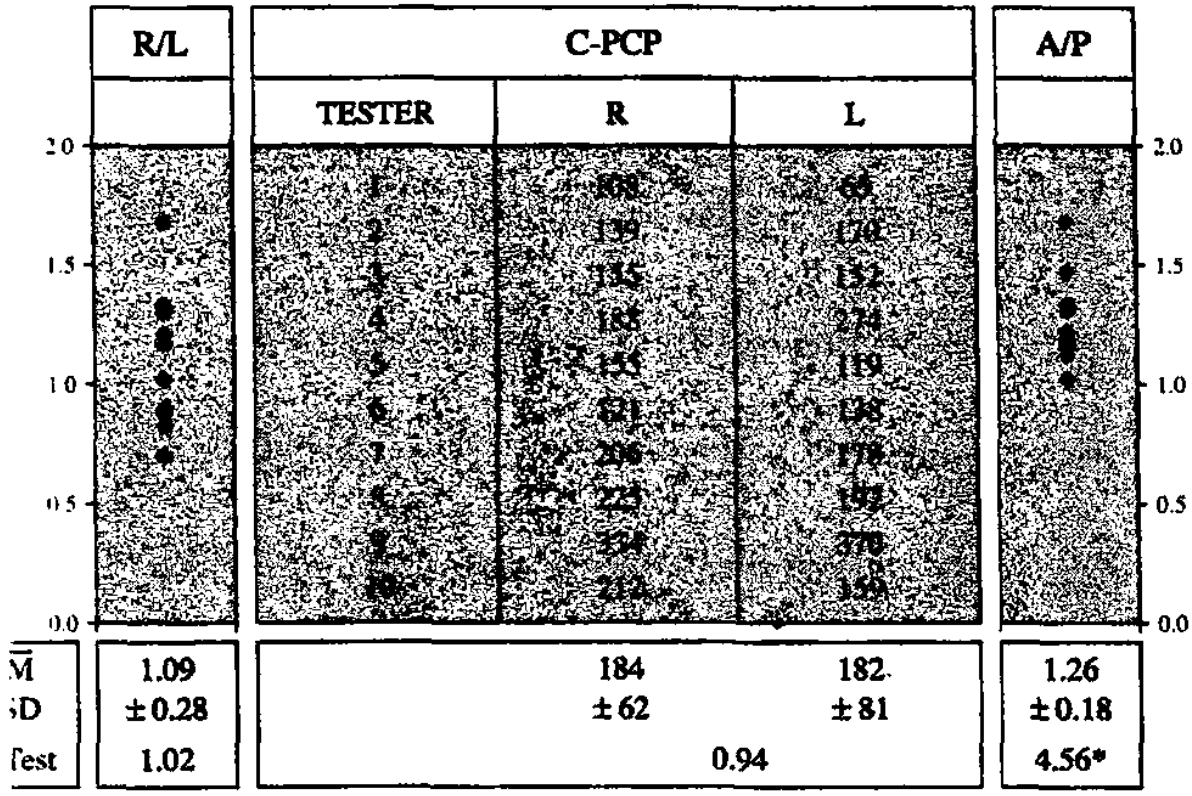
Student t-test:
- bilateral for means;
- unilateral for ratios.

Results

Results 1

The studied population is heterogeneous in terms of steady state level of m-RNas.

Only a comparative analysis of the active (A) on one side, placebo (P) on the other side in the same subject can reveal significant differences:
- The mean values (M) are similar for the right (R) and the left (L) sides.
- The ratios (R/L) are equally distributed around 1.0: the difference between R and L is not significant.
- The values of A shaded) are all higher than those of P (ratio A/P higher than 1.0) and the difference is highly significant (*$p < 0.01$).



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Results 2

The collagen m-RXAs are increased on the side treated by vitamin C (A) by comparison to placebo (P) similarly for α1 I and α1 III.

The ratio between the number of copies of α1 I and α1 III per arbitrary unit of 2SS r-RNA is similar to that found in skin and is not disturbed by vitamin C.

	α1 I	α1 III	I / III	
TESTER	A/P	A/P	A	P
1	1.66	1.34	2.91	2.45
2	1.38	1.64	2.58	3.07
3	0.92	0.91	2.84	2.75
4	1.55	1.56	3.01	3.02
5	1.74	1.59	2.72	2.49
6	0.89	0.96	2.59	2.80
7	1.24	1.24	2.55	2.56
8	0.71	0.62	3.02	2.64
9	0.83	0.78	2.76	2.57
10	1.62	1.45	3.04	2.72
$\bar{M}$	1.25	1.21	2.80	2.71
t-Test	p < 0.01	p < 0.05	0.19	0.21

Results 3

The steady-state level of the processing (N-PCP and C-PCP) and crosslinking (LO) enzymes is increased on the side treated by vitamin C (A) by comparison with the side treated with placebo (P).

	N-PCP	C-PCP	LO
TESTER	A/P	A/P	A/P
1	1.33	1.66	5.67
2	1.10	1.22	2.53
3	0.87	1.02	0.57
4	1.96	1.46	1.18
5	1.58	1.30	2.20
6	1.19	1.14	1.41
7	1.00	1.16	2.55
8	0.86	1.17	0.24
9	0.79	1.11	1.47
10	1.41	1.33	1.45
$\bar{M}$	1.21	1.26	1.93
t-Test	p < 0.05	p < 0.01	p < 0.05

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Results 4

The steady-state level of the non specific markers of the dermal cells (Vim) and of keratinocytes (K10) is increased by the topical application of vitamin C. But the ratio Vim/K10 is not modified by treatment with the active (A) preparation compared to the placebo (P).

	K10	VIM	VIM/K10	
TESTER	A/P	A/P	A	P
1	1.58	1.88	7.22	6.05
2	2.64	1.29	6.88	[14.10]
3	0.93	0.87	5.27	5.63
4	0.90	1.53	4.53	2.68
5	1.17	1.38	4.82	4.18
6	1.68	1.03	2.65	4.31
7	1.88	1.06	3.59	6.34
8	1.17	0.83	3.77	5.36
9	1.23	0.88	3.21	4.48
10	1.46	1.24	4.19	4.93
$\bar{M}$	1.46	1.20	4.61	4.88
t-Test	$p < 0.01$	$p < 0.05$		

Results 5

The steady-state level of the MMPs is not significantly modified by the topical application of vitamin C. MMP1 is barely and irregularly expressed (not illustrated).

	MMP2	MMP9
TESTER	A/P	A/P
1	3.63	0.31
2	1.94	0.67
3	0.70	0.35
4	0.66	0.99
5	2.59	2.11
6	1.24	1.50
7	2.39	2.42
8	0.19	0.67
9	0.73	2.05
10	0.99	2.99
$\bar{M}$	1.51	1.41
t-Test	NS	NS

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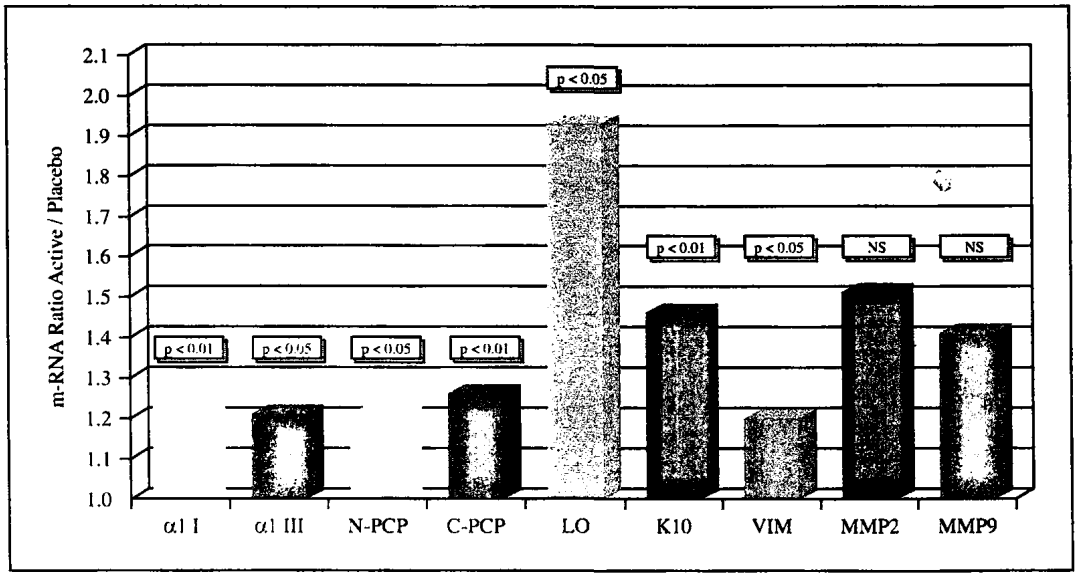
Changes in the levels of m-RNA coding for collagen, postranscriptional processing enzymes and markers of epidermal and dermal ceUs activity following 6 months treatment with topical vitamin C. (Summary of the results)



Conclusions

1. Topical vitamin C in a w/o emulsion stimulates the synthetic activity of the dermal and epidermal cells.

2. The absence of dermal cell stimulation in 20 to 30% of the testers might be related to an optimal dietary saturation of the tissues in the vitamin.

3. The resorbed vitamin C can be expected to increase protection against free radicals.