Oral isotretinoin in photoaging: clinical and histopathological evidence of efficacy of an off-label indication

RM Rabello-Fonseca,*† DR Azulay,† RR Luiz,‡ CA Mandarim-de-Lacerda,¶ T Cuzzi,§ M Manela-Azulay†

† Department of Dermatology, Federal University of Rio de Janeiro, Brazil
‡ Institute of Public Health Studies, Federal University of Rio de Janeiro, Brazil
¶ Laboratory of Morphometry, University of the State of Rio De Janeiro, Brazil
§ Department of Pathology, Federal University of Rio de Janeiro, Brazil

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*Corresponding author, Department of Dermatology, Federal University of Rio de Janeiro, R. Professor Rodolpho Paulo Rocco, 255, 5° andar, Cidade Universitária, Ilha do Fundão, Rio de Janeiro 21941 913, Brazil,
tel./fax + 55 21 2562 2580;
E-mail: rosarabellof@gmail.com

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Abstract

Background Despite evidences of the beneficial clinical effects of oral isotretinoin in the treatment of cutaneous photoaging, scientific evidences are still scarce, mainly supported by histopathological and morphometric studies.
Objectives To analyse possible clinical and morphological changes resulting from the treatment of photoaging with oral isotretinoin.
Methods Thirty female patients, aged 40 to 55 years, phototypes II to IV, with moderate to severe photoaging were randomly assigned to two groups of 15 each. Group I (G I) patients were treated with 10 mg of isotretinoin and group II (G II) with 20 mg of oral isotretinoin thrice a week for 3 months. Skin biopsies were performed before and after the end of therapy, and the various sections were submitted to specific staining for collagen and elastic fibres. To analyse the changes, morphometric studies were performed, and the results obtained were analysed by Student's *t*-test (paired and non-paired). Clinical results of therapy regarding texture, colouring and aspect of the wrinkles were assessed by both physician and patient.
Results The increase in the amount of collagen fibres was statistically significant with both dosage regimens (mean, 37.8%, increasing to 44.4%; $P = 0.029$ with the 10-mg dosage; and mean, 36.6%, increasing to 41.9%; $P = 0.01$ with the 20-mg dosage). A pattern pointing toward a decrease in the number of elastic fibres was found (mean, 15.3–12%; $P = 0.014$ with the 10-mg dosage; mean, 15.5–14%; $P = 0.125$ with the 20-mg dosage). Additionally, there was improvement in the general aspect of the skin, regarding texture, wrinkles depth and skin coloration.
Limitations Despite ethical considerations, a lack of a control group using placebo may render the results less accurate.
Conclusion Low dosages of oral isotretinoin seem to be an effective therapeutic option for cutaneous photoaging.

Topical retinoids are largely used in the treatment of cutaneous photoaging, and their efficacy is well established. Kligman *et al.*, in 1986, compared topical tretinoin at a 0.05% concentration to placebo in patients with moderate photoaging and observed an improvement in clinical appearance, besides recovery of structural damages in the epidermal and dermal layers of the skin in patients receiving topical tretinoin.[1] The use of systemic retinoids aiming to reverse skin damage provoked by sun exposure has been receiving special attention in the past years. Oral isotretinoin has been prescribed in low doses for the treatment of cutaneous photoaging by some healthcare professionals with satisfactory clinical results.[2] In 2000, Hernandes-Perez *et al.* published a paper reporting that when acne vulgaris was treated with oral isotretinoin, their patients showed a global improvement

in the skin and not only an improvement of acne. From 1992 onwards, they started to use oral isotretinoin for rejuvenation obtaining satisfactory clinical results.[3]

Few existing studies emphasize only the clinical aspects[2,3] and do not demonstrate, through objective complementary techniques, the effects of systemic isotretinoin in skin remodeling.

Isotretinoin (13-*cis*-retinoic acid) is a first generation retinoid and, as all others, acts similarly to vitamin A.[4–6] Because its therapeutic index is superior to its isomer, retinoic acid (RA), its utilization revolutionized acne treatment. Its use has raised the interest of dermatologists new indications, including photoaging, have raised great expectation in clinical practice.[5–9]

This study aims to analyse the clinical and histological effects of the 10- and 20-mg dosages of oral isotretinoin on the photodamaged skin.

Materials and methods

Study design

Randomized, double-blind study to assess and compare the effects of 10 and 20 mg of oral isotretinoin given thrice a week for a 3-month period in patients with cutaneous photoaging. The protocol has been submitted for approval of the Ethics Committee of Hospital Universitário Clementino Fraga Filho, from UFRJ, and was approved in September 2004.

Patients

Thirty female healthy volunteer patients, phototypes II to IV, aged between 40 and 55 years clinically diagnosed with moderate (II) or severe (III) cutaneous photoaging according the Glogau[10] score for photoaging and in postmenopausal status or using a safe contraceptive method participated in the study.

Exclusion criteria included patients for whom the use of oral isotretinoin was contraindicated, body weight inferior to 50 kg, pregnant or nursing women, history of hypercholesterolemia, ischaemic heart disease, skeletal disease and other dermatological diseases. Patients submitted to any other aesthetic procedure like peeling, laser, intense pulsed light, dermabrasive therapies or having used any anti-aging cream within the past 6 months could not participate in the study. During the treatment period, only oral isotretinoin was administered, and the use of any topical product, including sun blocker, was contraindicated. Patients included in the study had a complete red and white blood count, liver function tests, lipid profile, creatine kinase, creatinine and βHCG performed prior to treatment and then monthly at each visit.

After receiving an explanation of the study project, each participating patient had to sign the consent form authorizing the publication of the results and the photographs taken.

Treatment

The population evaluated was randomly assigned into two groups:

Group I (G I): Patients receiving 10 mg of oral isotretinoin thrice a week for 3 months.

Group II (G II): Patients receiving 20 mg of oral isotretinoin thrice a week for 3 months.

Clinical assessment

Prior to the initiation of drug intake, a complete medical history directed to the current and past history of sun exposure, current use of topical and systemic drugs, previous aesthetic treatments and pre-existing diseases was taken.

Patients' photoaging status was classified according to the Glogau score.

After 3 months of treatment, a new clinical assessment was performed, and a score from 0 to 10 rating the improvement obtained with treatment was given both by the treating physician and the patient. The physician evaluation was obtained first, without the knowledge of the patient. Scores from 0 to 3 were considered as 'no improvement', from 4 to 6 as 'average result', from 7 to 8 as 'good', and scores 9 and 10 as an 'excellent result'.

Photographic assessment

Digital photographs and those taken with an ultraviolet (UV) filter were performed before and after treatment.

Histopathology

A sample of skin was obtained by a 5-mm *punch* taken from the left preauricular region of each patient before treatment and from the contralateral region at the end of the study. The material was preserved in buffered 10% formaldehyde and stained by haematoxylin-eosin and picro-sirius red for the analysis of collagen fibres and oxidized and non-oxidized resorcin–fuchsin for the analysis of elastic fibres.

Morphometry

Digital images were obtained from each subject (JPEG format, 36-bit colour, 1280×1024 pixels) with a LC Evolution camera and Olympus BX51 microscope. The

images were analysed with the program *Image Pro Plus* version 5.0 (Media Cybernetics, Silver Spring, USA) and then segmented by using the same level of semitones to obtain a uniform pattern of colour to measure the amount of collagen and elastic fibres pre- and post-treatment.

The *pixels* corresponding to the collagen fibres (plates attained with picro-sirius red) and to the elastic fibres (plates stained with oxidized and non-oxidized resorcin–fuchsin) were selected. The histogram of the segmentation of the images was evaluated, and the results of the selected *pixels* were expressed as a percentage of the total area of the image.

Statistical analyses

The results were evaluated by means of adequate statistical tests for each variable analysed. The chi-squared test was used to analyse the characteristics of both groups before treatment, such as phototype, level of photoaging according to the Glogau score and age. To assess the effects of treatment within each group, the paired *t*-test was used as well as the calculation of the 95% confidence interval (95% CI) for pre- and post-treatment variation. The comparison of the variation between the two groups was obtained by means of Student's *t*-test, and the 95% CI was calculated for the difference between them.

Results

Of the 30 initially selected patients, only one from G I was excluded from the study in the second month of treatment due to dyslipidaemia. All other patients concluded the study and did not present any significant clinical or laboratorial side effects.

By comparing the populations of G I and G II (Table 1), we observed that the randomization was effective, and the differences of the characteristics, regarding the phototype and photoaging found between the groups were non-significant from a statistical point of view. Regarding the variable age, mean age was 46.0 for G I and 45.2 for G II, revealing a balance between the two groups.

According to the clinical assessment with regard to the level of improvement, considering the opinion of the investigator and the patient, no patient from G I and only one patient from G II considered that there was no improvement with the treatment. Five patients in each group (i.e. 34.5% of the patients) on average considered the result of the treatment as excellent. The remaining patients from both groups considered the level of improvement as average or good.

According to the investigators' assessment, none of the patients had no improvement nor excellent response to treatment, all the results being considered average or good. Through mutual consent between the physician and the patient, a satisfactory agreement rate regarding improvement was observed between the two. In those patients in which the physician considered clinical improvement as being average, the patients were also found to consider improvement average or good. In those patients in which the physician found the results as being good, patients also found the results as being good or excellent. This was the case in both groups (Table 2).

An improvement of the aspect after the treatment was obtained in the majority of patients and could be detected

Table 1 Pre-treatment phototype and photoaging grade according to the 10- and 20-mg dosages

	Total		Group				
			10 mg (*n* = 14)		20 mg (*n* = 15)		
Characteristics	***n***	**%**	***n***	**%**	***n***	**%**	***P*-value of χ^2 test**
Phototype							
II	10	100.0	4	40.0	6	60.0	0.224
III	12	100.0	8	66.7	4	33.3	
IV	7	100.0	2	28.6	5	71.4	
Glogau score							
II	10	100.0	3	30.0	7	70.0	0.153
III	17	100.0	9	52.9	8	47.1	
IV	2	100.0	2	100.0	0	0.0	

Table 2 Clinical assessment regarding the level of improvement according to investigator and patient

			Result according to the investigator			
	Total		Average		Good	
Result according to the patient	***n***	**%**	***n***	**%**	***n***	**%**
Group 10 mg						
No improvement	0	0.0	0	0.0	0	0.0
Average	3	21.4	3	21.4	0	0.0
Good	6	42.9	1	7.1	5	35.7
Excellent	5	35.7	0	0.0	5	35.7
Total	14	100.0	4	28.6	10	71.4
Group 20 mg						
No improvement	1	6.7	1	6.7	0	0.0
Average	2	13.3	2	13.3	0	0.0
Good	7	46.7	5	33.3	2	13.3
Excellent	5	33.3	0	0.0	5	33.3
Total	15	100.0	8	53.3	7	46.7

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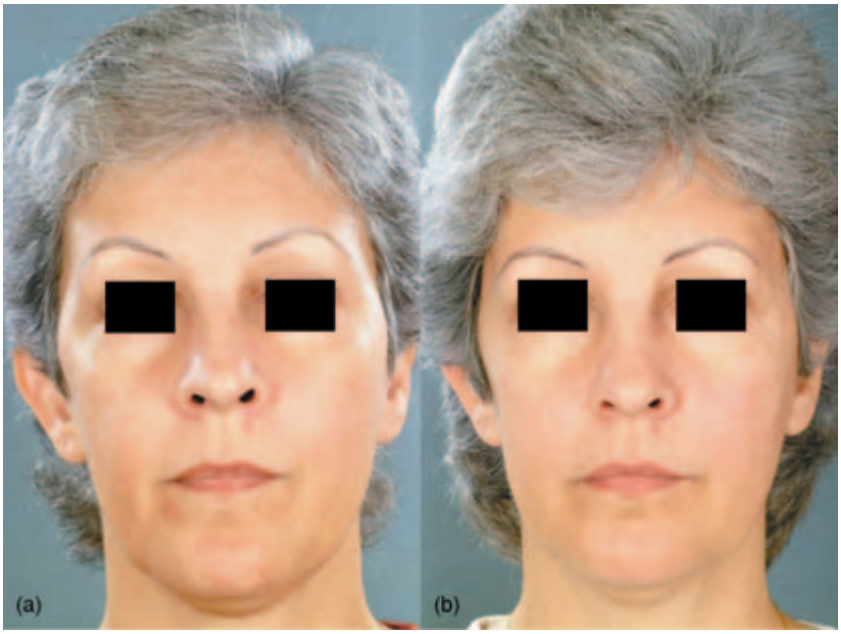

fig. 1 Patient reciving 20 mg of oral isotretinoin three times a week for three months. (a) Before treatment; (b) After treatment.

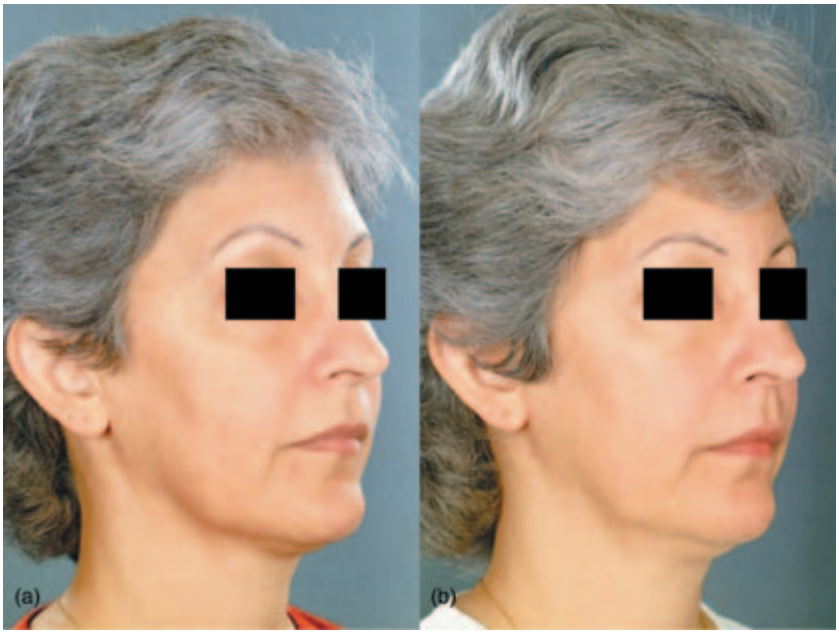

fig. 2 (a) Before treatment. (b) After treatment.

by clinical examination and through the photographs. The major clinical changes observed after treatment were decrease of flaccidness, smoothening of rhytids and wrinkles, pore minimization and improvement of skin texture (figs 1, 2, 3, 4).

Digital photographs with an UV filter have shown in many cases, decrease of pigmentation after treatment (fig. 5).

Before treatment, the histological sections stained by haematoxylin–eosin showed, in the majority of cases, non-specific inflammatory changes such as mild to moderate mononuclear inflammatory infiltrate mainly consisting of lymphocytes localized around the vessels and in neighbouring pilosebaceous structures, besides basophilic degeneration of collagen fibres, discrete in some cases but very important in others, forming agglomerates of

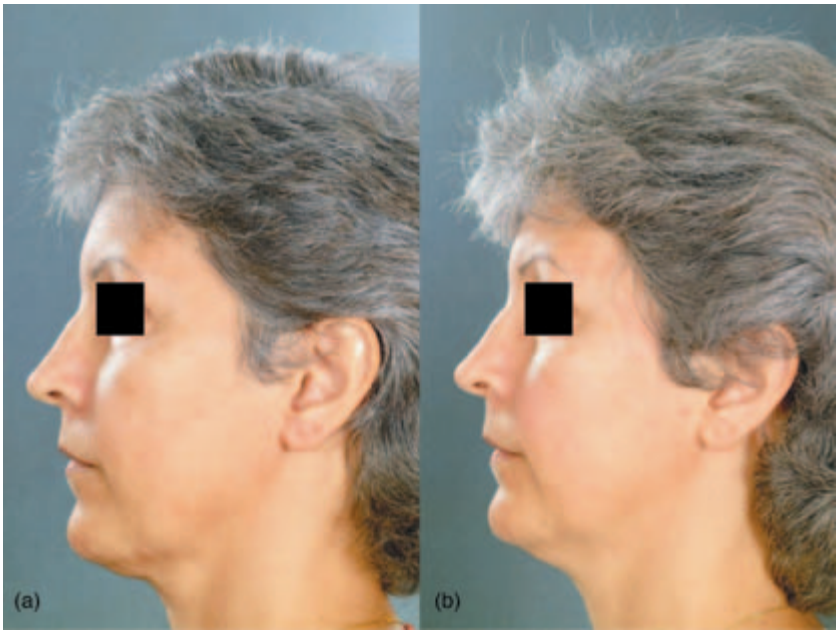

fig. 3 Side view. Notice the improvement in the submentonian and periorbital flaccidness. (a) Before treatment. (b) After treatment.

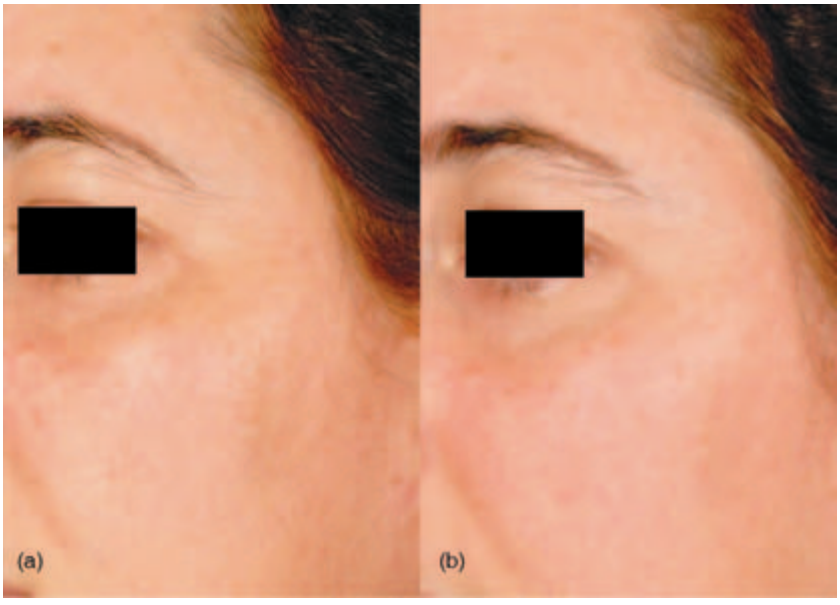

fig. 4 (a) Before treatment. (b) After treatment with 10 mg of oral isotretinoin three times a week for a 3-month period. Improvement of the texture, minimization of pores, lessening of periorbital rhytids.

amorphous basophilic material. After treatment, a discrete reduction of the inflammatory infiltrate and a reduction of the basophilic degeneration of the collagen were observed in the majority of cases.

The histologic sections of the skin stained by picro-sirius pre- and post-treatment were compared, and in most cases, an increase of the thickness of the derm layer was observed due to an increase in the amount and thickness of collagen fibres (fig. 6).

In general, we observed a decrease of the elastic fibres and elastotic material, when analysed by oxidized and non-oxidized resorcin–fuchsin stain, besides changes in the morphology of elastic fibres, more tangled and fragmented before treatment and more fibrilar and whole after treatment. The cited changes were more evident in five cases in which the severity of solar elastosis was more pronounced (fig. 7).

Though we found on optical microscopy changes suggesting an improvement of photoaging with therapy, we consider that the histopathological changes were discrete in some cases and difficult to assess leading us to make a more objective analysis. In light of this, the

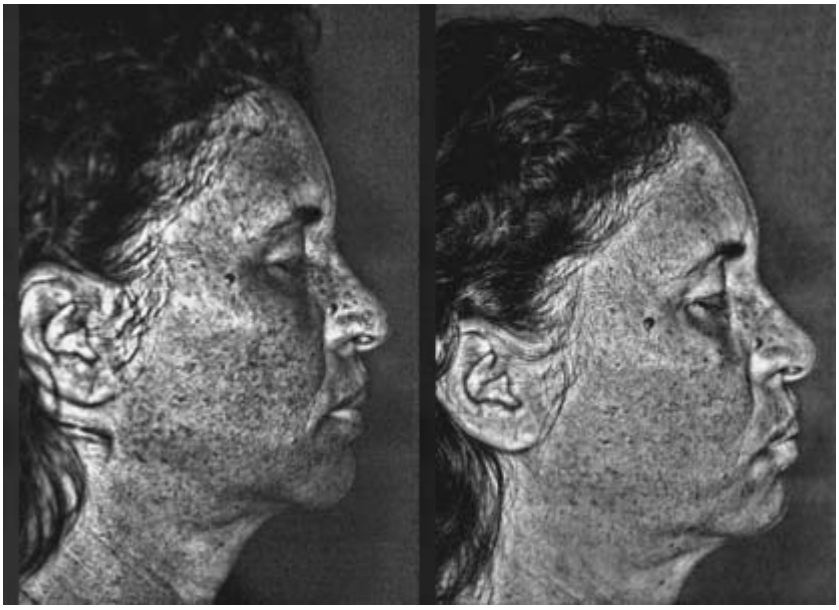

fig. 5 Photos with UV filter (a) Patient before treatment. (b) Three months after 20 mg of oral isotretinoin three times a week.

Table 3 Morphometric assessment pre- and post-treatment for the picro-sirius staining

	Groups							
	G I (*n* = 14)		**G II (*n* = 15)**		**Difference between G I and G II**			
	Mean	**Standard error**	**Mean**	**Standard error**	**Mean**	**Standard error**	**95% CI%**	***P*-value of the *t*-test**
Before	37.8	2.6	36.6	2.5	1.2	3.6	(–6.2; 8.5)	0.746
After	44.4	2.5	41.9	3.1				
Difference	6.6	2.7	5.3	1.8	1.3	3.2	(–5.3; 7.8)	0.691
95% Confidence Interval (CI)	(0.8; 12.4)		(1.5; 9.1)					
P value of the paired *T* test	0.029		0.010					

histological sections stained by picro-sirius and non-oxidized resorcin–fuchsin were subsequently evaluated by morphometry.

Through morphometric assessment, we observed a significant increase of the concentration of collagen after the treatment with both dosages. With the 10-mg dosage, the mean collagen concentration was 37.8% before treatment reaching 44.4% post-treatment. The difference between the means before and after treatment was 6.6%, being statistically significant ($P = 0.029$). With the 20-mg dosage, the mean collagen concentration was 36.6% reaching 41.9%. The difference between means before and after treatment for this group was 5.3%, also statistically significant ($P = 0.010$). The difference in the results obtained between the groups was not large enough to detect a statistically significant difference (Table 3; figs 8 and 9).

The morphometric assessment of the non-oxidized resorcin–fuchsin staining did show a decrease in the concentration of elastic fibres for both administered dosages (10 and 20 mg). The decrease was statistically significant for the 10-mg dosage ($P = 0.014$) but not for the 20-mg dosage ($P = 0.125$). Nonetheless, when the Student's *t*-test was applied to assess the difference between the results obtained with the 10- and 20-mg dosages regarding the decrease of fibres of the elastic system, we conclude that the difference was not statistically significant (Table 4; figs 10 and 11).

Discussion

The results of the present study point towards a trend that oral isotretinoin at dosages of 10 and 20 mg thrice a week

Table 4 Morphometric assessment pre- and post-treatment for the non-oxidized resorcin-fuchsin staining

	Groups							
	G I (*n* = 14)		G II (*n* = 15)		Difference between G I and G II			
	Mean	Standard error	Mean	Standard error	Mean	Standard error	95% CI	*P*-value of the *t*-test
Before	15.3	1.5	15.5	1.9	–0.2	2.4	(–5.2; 4.7)	0.923
After	12.0	1.3	14.0	1.6				
Difference	–3.3	1.2	–1.5	1.0	–1.8	1.5	(–4.8; 1.3)	0.249
95% confidence interval (CI)	(–5.8; –0.8)		(–0.5; 3.6)					
P-value of the paired *t*-test	0.014		0.125					

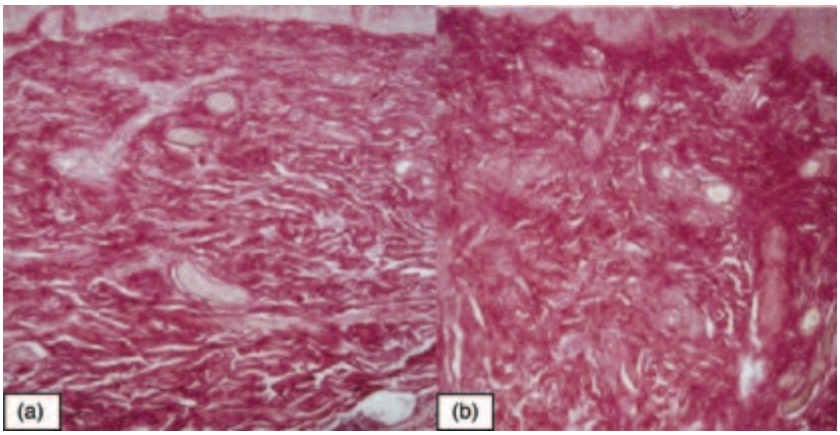

fig. 6 Histological sections of the skin stained by picro-sirius red where the collagen fibers are stained in red. (a) Before treatment. (b) After treatment with 20 mg of oral isotretinoin, showing an increase of collagen fibers within the superficial derm layer.

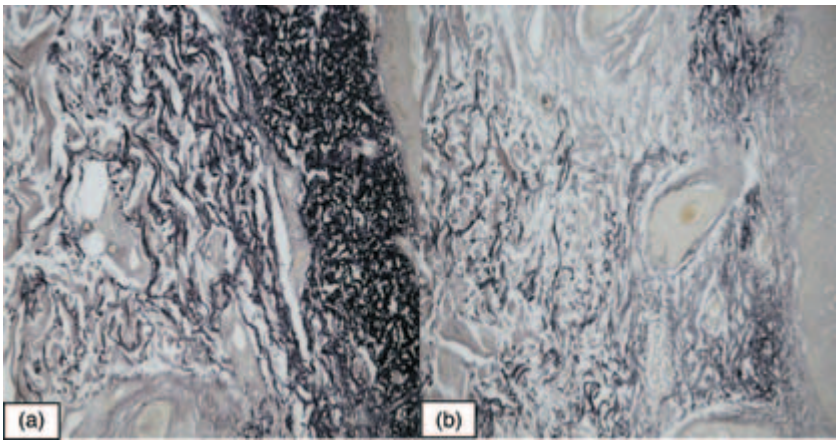

fig. 7 Non-oxidized resorcin-fuchsin staining. (a) Before treatment; elastotic material stained in purple; (b) After treatment with 10 mg of oral isotretinoin, showing decrease of elastotic material.

for a 3-month period is an effective and safe treatment for photoaging.

Non-randomized studies with no histopathologic assessment have been performed previously, demonstrating only clinical improvement in patients with photoaging.[2,3]

The present study was the first randomized clinical trial with clinical, histopathological and morphometric assessments following oral isotretinoin for photoaging showing an improvement of the general aspect of the skin and a significant increase in the amount of collagen fibres after the treatment with both dosages. Oral isotretinoin has also acted in the fibres of the elastic system, showing a trend in diminishing the actinic elastosis.

It's known that in the skin exposed to sunlight, especially in light-skinned persons, there is a decrease in collagen fibres and hyperplasia of elastic fibres with

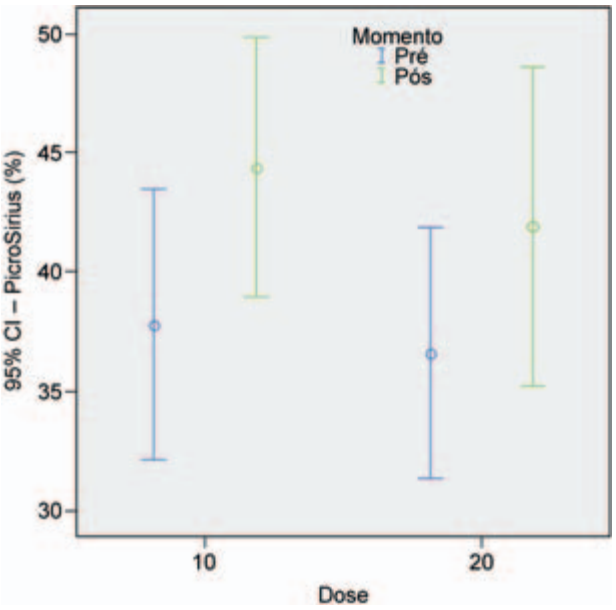

fig. 8 Bars graph illustrating the percentage of collagen fibers before (blue lines) and after the treatment (green lines) for the two dosages.

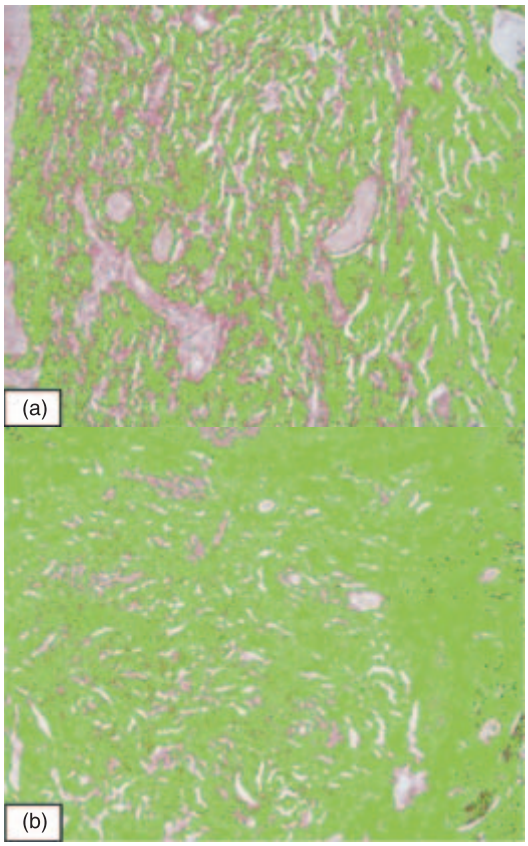

fig. 9 Histological sections stained by picro-sirius, where the collagen fibers are stained in green (morphometry technique). (a) Before treatment. (b) After treatment with 20 mg of oral isotretinoin.

ageing, being evident upon histopathological examination. The number of elastic fibres increases and they become thick, rolled up and tangled.[11–13] In patients with clinically evident actinic elastosis, the staining with haematoxylin–eosin reveals, in the upper derm, basophilic degeneration of collagen. With specific stainings for elastic fibres, not only the normal elastic fibres stain but also areas of collagen fibres and degenerated elastic fibres called elastotic material.[11,12,13]

Several topical treatments with retinoids,[1,9,14,16] beta hidroxiacids,[9,17] vitamin C,[18,19] vitamin E[20] and procedures such as chemical *peelings*,[21] dermabrasion[22,23] and ablative[22] and non-ablative[24] laser may be used to treat the photo-aged skin. The mechanism of action of these methods is related to an increase in the production of collagen, none of them changing the accumulation of deposited elastotic material[13] as observed in the present study with oral isotretinoin.

The method used to quantify the changes found after oral isotretinoin administration was morphometry.[25,26] This technique aims to quantify the amount of collagen and elastic fibres before and after treatment. It's worthwhile to note that it's an ethical procedure of assessment because the investigator interferes very little in the final result, obtained by means of software.

The idea of using the 10- and 20-mg dosages was to compare the clinical and histological effects, aiming to achieve the minimum effective dosage allied to a superior safety profile. According to the results, we observed that there are no significant differences between dosages, even

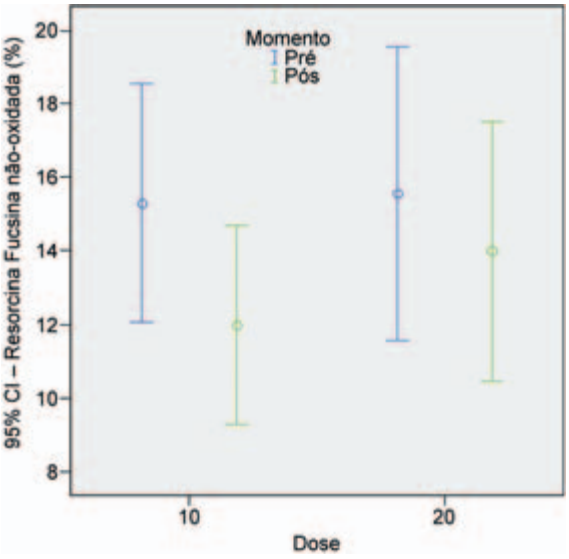

fig. 10 Bars graph illustrating the percentage of elastic fibers before (blue lines) and after the treatment (green lines) for the two dosages.

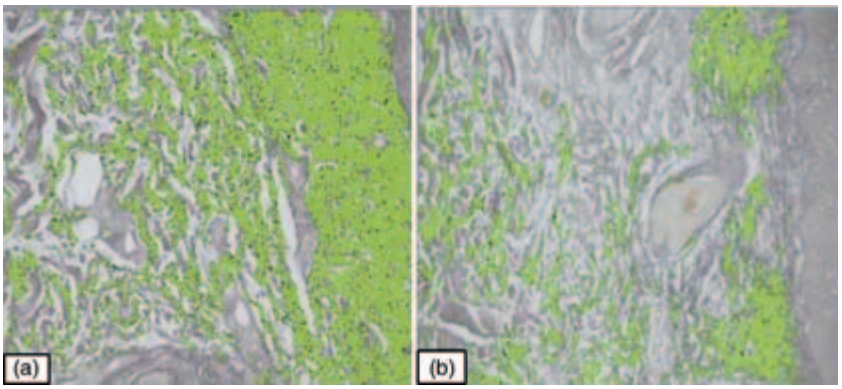

fig. 11 Histological sections stained by non-oxidized resorcin-fuchsin, where the elastic fibers are stained in green (morphometry technique). (a) Before treatment. (b) After treatment with 10 mg of oral isotretinoin.

suggesting that the 10-mg dosage could offer a better result. This, however, remains to be confirmed through further studies. It is tempting to speculate that the 5-mg dose could probably present with adequate results, something that should be subject to further evaluation in the future.

We decide to make the biopsy in the left side before and in the right side after the treatment which may lead to some kind of concern, once the right side of the face would be probably more prone to skin damage as a result of stronger sunlight exposure 'from driving with the window down'. But all patients in our study were from working class families and it was carried out in a public university hospital where the patients have no access to their own private transport. They usually use public transport such as bus or train. Therefore receive UV exposure from both sides, as they can seat in the left or in the right side randomly.

The mechanism of action of oral isotretinoin in photoaging remains to be clarified. As all topical retinoids, it probably acts by inhibiting metalloproteinases of the extracellular matrix, leading to an increase of collagen production, especially types 1 and 3.[27] Furthermore, we also noticed an activity on the elastic system not reported up until now. New studies using immunohistochemical techniques, electronic microscopy and fibroblast culture are necessary to better define how isotretinoin acts on the fibres of the elastic system as well as which subtype of fibre decreases or increases. Despite the fact that photoaging also affects patients of an older age range, for this specific study, we chose only patients aged between 40 and 55 years. We adopted such strict standards of inclusion due to the fact that older patients generally take multiple medications on a regular basis and therefore possess various comorbidities. Thus, although we cannot state that this specific kind of treatment does not function for older patients, we are able to suggest that treatment would indeed be more appropriate for patients of a younger age range for the above-mentioned reasons. Although we recognize the importance of the placebo, particularly in this study, besides the ethical involvement, the placebo could be dispensed without any negative consequences for our conclusion. It is reasonable to think that, as ageing skin is an inexorable dermatologic problem that worsens day by day, the observation of improvement after the treatment, corroborated by the morphological changes observed in this study, point without doubt that the improvement was caused as a result of medication.

This pioneer study demonstrated that oral isotretinoin is beneficial to the extracellular matrix remodeling process. Nonetheless, additional studies are necessary, especially aiming to clarify the mechanism of action of oral isotretinoin on the components of the extracellular matrix and the duration of the effects obtained. Studies comparing topical and oral retinoids would be of great value for the scientific community, in order to evaluate the costs and benefits for each indication.

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