Original Article

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Microcirculatory and clinical parameters in patients with gynoid lipodystrophy treated with topical garlic (15PPM)

ABSTRACT

Background: Gynoid lipodystrophy, popularly known as cellulite, is regarded as an issue of cosmetic concern to millions of women. Recent studies have shown that garlic extract can be useful to enhance lipolysis and decrease lipogenesis. Objective: The primary objective was to determine if topical application of a lotion containing garlic (15ppm) would improve cellulite parameters. Methods: 50 women were treated topically with a lotion containing 15ppm of garlic. All patients treated one leg, did not treat the other one and were evaluated (measurement of thigh diameter) before and in biweekly intervals up to 8 weeks after the onset of treatment. On eight of these subjects, microcirculatory parameters [red blood cell velocity in arterioles and venules (mm/s), functional capillary density (FCD), diameter of the dermal papilla (μ m) and capillary diameter (μ m)] were measured using the Cytoscan[®]. The exact thigh local for these measurements was marked in a transparent sheet, to assure that all of them were performed on the same place. Six women that performed the Cytoscan[®] exam group were also evaluated by ultrasound to determine the thickness of subcutaneous tissue. Results: after garlic treatment, there was a reduction of 1.62 cm on the diameter of the treated thigh (p<0.001) without significant changes on papilla and capillary diameters, but FCD improved significantly (p < 0.03) and epidermis and dermis thickness decreased after topical garlic treatment. Conclusions: Garlic extract at a concentration of 0.0015% showed significant clinical, microcirculatory and ultrasonographic improvement of cellulite.

Keywords: garlic extract, cellulite, microcirculatory parameters.

INTRODUCTION

Gynoid lipodystrophy, popularly known as cellulite, is considered an issue of cosmetic concern to millions of women^{1, 2}. It is characterized by orange peel or cottage cheese-type dimpling of skin seen most commonly on thighs and buttocks^{3,4}. Cellulite can be found in any area of the body that contains subcutaneous adipose tissue. However, certain areas are more susceptible than others are, such as upper outer thighs, posterior thighs and buttocks. Cellulite affects 85-98% of post-pubertal females of all races.

Various methods have been described to try to "treat" cellulite. Despite numerous therapeutic modalities, there is little scientific evidence that any of these treatments are beneficial. Some of the few studies have used only thigh measurement and photography to assess improvement, both far from accurate and reproducible.⁵

The garlic extract (*Allium sativum* sp) has been proposed as an useful treatment in obesity and localized fat, included in popular literature as well as in popular beliefs⁶. The extract contains several active principles. Studies carried out *in vitro*, have shown that molecules in the extract have suppressor or depressor effect on several enzymes related to lipogenesis, such as PPAR 2, LPL, LHS and GLUT4, in addition to a probable negative effect on leptine production⁷.

Garlic extract in a concentration of 0.0015% may also increase the expression of LHS, UCP1 and UCP2 molecules related to adipocytes and phenotypically prone to oxidize

(We declare no conflict of interest).

fat (lipolysis) rather than store it⁸. Therefore, garlic extract would be able to reduce the storage of adipose fat by reducing the expression of molecules related to lipogenesis (leptine, PPAR 2, LPL, GLUT4) and also by increasing expression of other molecules more related to lipolysis (LHS, UCP1 and UCP2).

Several imaging technologies to facilitate visualization of cellulite have been described, USG is a well-known method to study dermal architecture and we have previously shown in our articles^{10, 11} that Orthogonal Polarization Spectral (OPS) imaging is very useful to study microcirculatory parameters in cellulite.

ORTHOGONAL POLARIZATION SPECTRAL IMAGING

The exact mechanism is better shown in our previous articles^{10,11}. Imaging of human microcirculation using reflected light has been limited to vascular beds where the vessels are visible and close to the surface (e.g. nailfold, conjunctiva). Direct observation of vascular beds of other organs in humans has been prohibitive because of toxicity (fluorescent dyes for contrast enhancement), or the size of the instrumentation required to acquire images (transillumination).

Using a similar basic technique described by Slaaf *et al.*, who reported on intravital microscopy technique with two orthogonal polarizers, Groner *et al.* developed a handheld portable instrument allowing easy access to a variety of vascular beds in patients. The Orthogonal Polarization Spectral Imaging (OPS) technique has been incorporated into the Cytoscan[®] (Cytometrics, Philadelphia, PA, U.S.A.)

In OPS imaging, the tissue is illuminated with linearly polarized light, wavelength of 538 nm reflected through a polarizer oriented orthogonally to the plane of the light. Since polarization is preserved in reflection, only photons scattered from relatively deep in tissue contribute to the images. Using Cytoscan[®], with special optics, we can create a virtual light source that penetrates 1 mm inside the tissue. When the light is absorbed by hemoglobin (Hb), an image of the illuminated Hb-carrying structures in negative contrast is created. This patented "virtual backlighting" technology let us visualize and measure real time images of the microcirculation without the use of fluorescent dyes or transillumination.

When reflected light is used, it is quite difficult to obtain a good image contrast and detail due to the surface scattering and the turbidity of the surrounding tissue. In OPS imaging the phenomenon of cross-polarization mitigates these effects. The method has been validated for quantitative measurements of microcirculatory parameters in animal models compared to intravital fluorescence microscopy^{9, 12}.

SKIN ULTRASOUND

Skin ultrasound (USG) of 10-12 MHz is a technique that has already gained recognition for the assessment of dermal and hypodermal structures¹³⁻¹⁵. Differences in echogenicity are useful for the assessment of the etiology of several skin neoplasias and, according to Cammarota and co-workers¹³, it is possible to differentiate the echogenic pattern of the intra-dermal melanocytic nerves from those observed in invasive melanomas of the dermis. Likewise, vascular structures are easily identifiable by means of this technique, an useful fact in surgical interventions of cavernous as well as tuberous hemangiomae. Cystic tumorlike lesions, such as sebaceous and dermoid cysts, may have their contents identified through USG, for keratin and osseous as well as cartilaginous embryo, structures that feature different echogenicity¹⁴.

With its pattern of dermal and hypodermal fibrous septa and intense dermal edema, gynoid lipodystrophy becomes susceptible to assessment by means of 10-12 MHz USG. The degree of tissue edema may be inferred by pattern comparison of dermal and hypodermal echogenicity, before and after treatment.

Effects of topical garlic extract in the treatment of gynoid lipodystrophy may be evaluated by measuring parameters of dermal microcirculation. The degree of tissue perfusion may be inferred by erythrocyte velocity in arterioles and venules, functional capillary density (number of capillaries with flowing red blood cells per unit area of tissue), and observation of the architectural pattern and symmetry of the vascular network distribution. Clinical and ultrasonographic assessment of patients could also be useful in the study of efficacy of topical garlic extract in the therapeutic handling of gynoid lipodystrophy.

OBJECTIVES

The General aim of the study was to determine using Cytoscan[®], ultrasound and thigh diameter measurements, the efficacy of a topical lotion containing 15ppm of topical garlic in the treatment of cellulite.

We can say that specifics objectives were to evaluate the following parameters, in treated versus non-treated areas: Changes on thigh diameter, changes of perivascular dermic edema, inferred by microcirculatory changes and thickness of the subcutaneous tissue.

MATERIAL AND METHODS

50 women were selected from the general population by aleatory amostrage. They were selected for treatment according to parameters of Fitzpatrick phototypes I to IV (Excess of melanin in patients with dark skin [Fitzpatrick phototypes V and VI], does not allow microcirculatory visualization with the OPS technique); Body mass index between 20 and 24; No diet during the study period and Age between 20 and 39.

Exclusion criteria were alteration in physical activity level during the evaluation; Gain or loss of more than 1.5 kg during the study; Use of other products on their legs except the one being tested; Interruption of treatment and; Miss visits during the study period.

Eight women of the group were evaluated by Cytoscan[®], and six of them also by USG.

Clinical and Microcirculatory Assessments: Each patient received flask-vials containing 20 ml of the product, enough to cover thigh and buttock on a daily basis. Each patient had her thigh diameter measured in the inclusion and in every visit thereafter.

Each patient was assessed 5 days (D0, D1-D4), and the first appointment was geared to the application of inclusion/exclusion criteria and of a questionnaire containing personal data (D0) and the other 4 (D1-D4), with biweekly intervals up to 8 weeks, for measurements of microcirculation as well as the clinical parameters. The onset of treatment took place on the second appointment (D1) with assessment by means of the OPS technique and thigh diameter measurement. Ultrasound and OPS exams were repeated 30 days after the beginning of the treatment (D2) and upon termination of it (D4).

Measurements of thigh circumference, visual and photographic grading were undertaken as previously described in an article of our group¹¹.

OPS Technique: The OPS technique was applied to treated skin to seek and evaluate the following microcirculation parameters¹², namely:

- Detection of red blood cell velocity in arterioles and venules;
- Functional capillary density;
- Diameter of the Papilla and of the Capillary (Figure 1) and
- Observation of the architectural pattern and of the distribution symmetry of the distribution.

The exact place on the thigh observed by the Cytoscan[®] was marked in a transparent sheet, to assure that all of measurements were performed at the same place. For this procedure, a chosen region, on the external face of the thighs, was selected and plotted on a plastic sheet used like a map. It was important to exclude possible alterations on the microcirculation of different areas. The first measurement was made on both legs and was used as a reference point. The OPS imaging probe was applied

to 8 separated round targeted areas at the edge of the previously marked area, for approximately 30 seconds each. Images of the microcirculation were video-recorded and stored into VHS format. The working distance of the OPS imaging probe, covered with a disposable sterile plastic cap, was approximately 3 mm. Sterile mineral oil was applied between the probe and the skin in order to improve coupling and, consequently, reliability of the readings. The analysis of selected parameters was performed using the CapImage[®] software¹⁶. This method was used exactly as seen in our previous study¹¹.

Assessment of the Areas Treated By Means of 10-12 MHz USG: 10-12 MHz USG was applied to the treated skin, and ultrasound images as well as reports were analyzed to compare D0 and D4 appointments, respectively, after 30 and 60 days of treatment. We performed measurements of thickness of the subcutaneous tissue.

Microcirculatory parameters were studied with Wilcoxon test to compare initial to final evaluation. Ultrasonographic parameters were evaluated using the W test of normality (Shapiro-Wilk).

RESULTS

From the first 50 patients selected, only 34 completed the protocol, 16 were excluded because they fall in one or more of the exclusions criteria during the protocol. 8 patients were examined with the Cytoscan and 6 were submitted to USG. Other patients were excluded based on above mentioned criteria. Results were as follows:

1. Clinical parameters

Clinical evaluation of the drug, made possible by diameter measurements of upper and lower thigh under treatment, as well as the contra-lateral one (control), showed a reduction in treated patients, on the two observed portions (Table 1). Median circumference reduction on thicker portions of treated thighs was 1.62cm (p<0.001). When considering the lower portion



Figure 1. Diameter of papilla and capilar by OPS Technique

Table 1. Diameter measurements (cm)										
		Mean	Standard deviation	Ν	Difference	Standard deviation of the Difference	t	L.G.	р	
Visit 1	Superior Treated Leg		3.07							
Visit 5	Superior Treated Leg	55.19	2.88	34	1.62	1.7	5.44	33	0.00001	*
Visit 1	PSuperior Not Treated Leg	56.79	3.26							
Visit 5	Superior Not Treated Leg	56.32	3.21	34	0.47	2.1	1.31	33	0.20	n.s
Visit 1	Inferior Treated leg	43.22	2.79							
Visit 5	Inferior Treated leg	42.32	2.31	34	0.9	1.8	2.96	33	0.01	*
Visit 1	Inferior Not Treated Leg	43.18	2.87							
Visit 5	Inferior Not Treated Leg	42.93	2.20	34	0.25	1.4	1.05	33	0.30	n.s
Visit 1	Weight	60.68	5.32							
Visit 5	Weight	60.86	5.30	34	-0.18	1	-1.03	33	0.31	n.s

ns= not significant

Table 2. Microcirculatory parameters

	Wilcoxon Test	Valid N	т	Z	р	
Diam. Papilla	Treated Leg D0 x Treated Leg D4	8	15	0.42	0.67	n.s
	Not Treated Leg D0 x Not Treated Leg D4	8	12	0.84	0.40	n.s
	Treated Leg D0 x Treated Leg D4	7	10	0.68	0.50	n.s
Diam. Capillary	Not Treated Leg D0 x Not Treated Leg D4	7	12	0.34	84 0.40 n 68 0.50 n 34 0.74 n	n.s
FCD	Treated Leg D0 x Treated Leg D4	8	10	1.12	0.26	n.s
	Not Treated Leg D0 x Not Treated Leg D4	8	12	0.34	0.74	n.s

ns= not significant

Table 3. Comparison treated x not treated leg								
	Wilcoxon Test	Valid N	Т	Z	р			
Diam. Papilla	Delta Treated Leg x Delta Not Treated Leg	8	8	1.40	0.16	n.s		
Diam. Capilar	Delta Treated Leg x Delta Not Treated Leg	7	13	0.17	0.87	n.s		
FCD	Delta Treated Leg x Delta Not Treated Leg	8	2	2.24	0.03	*		

FCD: Functional Capillary Density; ns= not significant

Table 4. Utrasonographic Results with Shapiro-Wilk Test.									
	Description	N of the Sample	Median [1o – 3o quartile]	W	р				
1 st visit	Thigh Epidermis	6	0.40 [0.40-0,50]	0.81	0.07	n.s			
1 st visit	Thigh Dermis	6	2.10 [2.00-2.20]	0.87	0.20	n.s			
5 th visit	Thigh Epidermis	6	0.40 [0.40-0.50]	0.70	0.01	0.01*			
5 th visit	Thigh Dermis	6	1.90 [1.60-1.90]	0.79	0.04	0.04 *			

ns= not significant

(10 cm above the patella), the difference between the first and last evaluation was 0.9 cm reduction, for treated thighs which was also significant (p<0.01).

All patients went through a complete dermatological exam on treated and non-treated areas, in each visit. They were also questioned about signs or symptoms of adverse reactions including allergic or irritant contact dermatitis. There was no evidence of adverse reaction, local or systemic, in any patient of the study.

2. Microcirculatory parameters

The diameter of the dermic papilla decreased when the interstitial edema decreased. Tables 2 and 3 demonstrate that it remained unchanged.

Functional capillary density (FCD) increased in a direct proportion to interstitial edema reduction, making it easier to visualize capillaries. In our subjects, the garlic solution significantly increased FCD in the treated leg when compared with the non-treated one, as we can see when the analyze (Table 3) were performed.

It is expected that the capillary diameter reduced with the decreased edema. However, there was no statistical significance between the two groups and in the capillary diameter of the treated and non-treated legs in patients.

3. USG parameters

The ultrasonographic parameters were evaluated with the W test of normality (Shapiro-Wilk). The results comparing the 1st visit and the 5th visit were a decrease in the W test both for the epidermis as for the dermis of the treated legs. These results (Table 4) were statistically significant (p < 0.05).

DISCUSSION

Evaluation of therapeutic modalities for cellulite is very difficult due to confounding factors, such as diet and exercise, as well as the absence of standard and objective criteria used to assess treatment response. Treatment categories include attenuation of aggravating factors, physical and mechanical methods, pharmacological agents and laser therapy^{11, 17-20}.

Pharmacological agents used for the improvement of cellulite include xanthines, retinoids, lactic acid and herbals. Even though there are several topical treatments that are available over-the-counter at drugstores, there are no large–scale studies demonstrating the effectiveness of any of these therapies. Only two of these drugs, aminophylline and retinoids²¹⁻²³, have been critically evaluated, with poor results, and one well evaluated with some results¹¹.

In our study with the OPS imaging system, the use of the garlic extract discretely improved the microcirculation.

A treatment with this garlic extract showed statistical significance concerning reduction of circumference and improvement of ultrasonographic parameters on treated thighs.

Weight loss, diet and regular practice of exercise are considered means of improving cellulite, even though there are no studies that confirm this theory. Many patients confuse weight gain with the appearance of cellulite. However, obesity or adipocyte volume alone does not create cellulite as seen in nearly all-lean females and very few obese males. On the other hand, weight loss does diminish the clinical perception of cellulite even if it does not alter the physiological reasons that created it^{1, 24-26}. In conclusion, garlic extract at a concentration of 0.0015% showed significant clinical and ultrasonographic improvement and discrete correlation to microcirculatory parameters after the treatment.

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