EFFECTS OF MICRO CURRENT, COLLAGEN, AND DMAE ON THECONNECTIVE TISSUE OF WISTAR RATS EVALUATED BY METHODSHISTOLOGICAL AND GRAVIMETRIC

ABSTRACT

Introduction. Skin aging is a degenerative process, involving intrinsic and extrinsic factors, being related to photoaging, oxidative stress, and inflammatory response, a process that induces the degenerative effect in the collagen network. In order to minimize skin aging, the intake of collagen, micro ~~corrent~~ currentand DMAE is an alternative in the aesthetic environment, however, the mechanisms of action involved in these phenomena are not fully understood. **Objective**. To evaluate the relationship between CM actions and direct collagen administration such as oral supplementation and DMAE, in the effectiveness or efficiency in the rejuvenation of integrodermal tissues in rats that did not suffer tissue injury, from their observed effects on connective tissue. **Material and Method.** The sample consisted of 12 Wistar rats (Rattus norvegicus), subdivided into four groups, respectively, control group (CG) that will not receive an application of micro current (CM); treatment-7 group (GT7), which will receive MC applications for one week once a day; collagen treatment group (GTCo), in which hydrolyzed collagen will be administered orally for 1 week (1 per day); and dmae treatment group (GTD), in which intradermal microinjection of DMAE will be applied. After treatment, the skin tissues of the animals were analyzed by histological techniques and by gravimetric method. **Results:** The GTCo presented an average area in µm2 and a higher percentage when compared to the control by analysis of variance with p: 0.044. **Conclusion:** The use of oral collagen for seven days in animals was efficient after histological analysis.

Keywords: Micro current, elastic fibers, collagen, and DMAE.

1. INTRODUCTION

Wrinkles are natural consequences of the aging process gradually arising due to looseness in the dermo-epidermal junctions and ~~the~~ modern advents focused on research on facial aging, ~~has~~ haveincited studies and ~~the~~ knowledge about the functional clinical aspects resulting from this process [1].

~~In senescence~~Senescence and/or cutaneous senility is described as a deteriorating and progressive factor of the collagen network, specifically affecting type I [2].

As a result of the loss of anchorage and the addition ~~with~~ ofelastic fibers ~~of~~ tothe dermis, there is a decrease in the tension of the collagen fiber network. With this, there is an increase in the stiffness of the tissues, which constitutes obstacles to the processes of transport and diffusion of nutrients from the blood capillaries to the tissue interstice, causing degradation of the cell functions ES [3]. What results from this pathophysiological process is that the skin loses natural elasticity, causing significant changes in collagen and elastic fibers, since the decrease in the activity of fibroblasts, significantly reduces the synthesis and consequent production of collagens [4, 5].

There are several therapeutic modalities in the area of dermato-functional physiotherapy specific for tissue repair, one of ~~them~~ their applications of micro currents, a resource used in the treatment of skin aging in aesthetic clinics [6, 7]. ~~Micro~~ The microcurrent seems to induce the ATP production phenomenon (adenosine triphosphate), responsible for protein synthesis and tissue regeneration, since its involvement in all cellular energy processes is proven [8,9], and there is a possible relationship between an increase in the number of fibroblasts and alignment of collagen versus CM fibers, with maximum fibroblast responses observed in the regions close to the cathode stimulation electrode.

In tissue lesions, there are reports that excitation by electrical means promotes an increase in the concentration of growth factor receptors, which can potentiate processes in collagen production [10, 11, 12].

This study aims to investigate the effects of CM in a smaller session of 7 applications in intact dermal tissue in rats that did not suffer tissue damage and histologically quantify possible counts in collagen and elastic fibers as possible formations of neo-collagens or greater amounts of elastic fibers and, also, to propose and test an alternative gravimetric method in place of histological techniques frequently used in the approaches of this theme. Therefore, its objective is to aim at a relationship between CM action and direct collagen administration such as oral supplementation and DMAE, in the effectiveness of age or efficiency in the rejuvenation of integrotemic tissues in rats that did not suffer tissue injury, from its observed effects on connective tissue.

2. MATERIAL AND METHODS

This ~~is an~~ experimental study using laboratory animals~~,~~ was approved by the Ethics Committee on the Use of Animals (CEUA) of Gurupi University - UnirG under opinion number 004/2021.

The sample consisted of 12 Wistar rats (Rattus norvegicus), originally from UnirGbioterium, randomly selected with the weight between 250 and 300 g, subdivided into four groups of 4 animals, respectively, control group (CG) that will not receive an application of micro current (MC); treatment-7 group (GT7), which will receive MC applications for one week once a day; collagen treatment group (GTCo), in which hydrolyzed collagen will be administered orally for 1 week (1 per day); and DMAE treatment group (GTD), in which intradermal microinjection of DMAE will be applied.

The animals were kept during the experimental protocols in the same environment of the bioterium, at an average temperature of 25 °C and controlled lighting with a photoperiod of 12 light hours/12 hours-dark and minimum noise, confined in cages lined with shavings and fed with commercial ration ad libitum, and the operational procedures were performed in the venous period. All rats were positioned in a polypropylene contensor~~in order to~~to minimize handling and promote animal stress.

The animals CG and GT7 were anesthetized with sodium thiopental at a dose of 50 mg/kg intraperitoneally, and in the rats of the CG, only simulations of the current applications were made with the device switched off. The rats of GT-Co (administered 0,5 mL of oral collagen daily for 7 days) and GTD (intradermal microinjection of 0,2% of DMAE (Dimethylaminoethanol) daily for 7 days) were not anesthetized.

In the MC group, the Ibramed ® (Brazil) type device was used, modulated to (parameters: 700 µA, 500 Hz, and 30 minutes/day), in which two electrodes with 4 cm² of the area were coupled to the trichotomized region using electrolytic gel, maintaining a distance of 5 cm, in sliding movements on the surface.

~~At~~the ~~At~~ theend of the experimental protocols, all 12 animals were sacrificed by thiopental overdose, diaphragmatic perforation, and injection of 0,1 mL of KCl 3 M into the heart, then skin samples with about 1 cm2 of the trichotomized surfaces were removed for histological analysis.

The tissue samples were placed in identified vials containing 20 mL of 10 % buffered formaldehyde solution. After the fixation process, the second routine protocol for the preparation of histological slides was processed, using hematoxylin-eosin (HE) stains to verify differences in collagen tissue in relation to the control.

Photographs were taken with the zensys trinocular microscope, and 10 photographs of histological slides of each rat were obtained, obtaining 30 images of each group. Subsequently, the images were analyzed by the J Image program to detect the percentage of collagen in each image. An average of 30 images were made to obtain the percentage of collagen per group.

3. RESULTS

After the time of the experiment was completed, the sacrifice of the experiment was performed, and the tissue was removed, processed, and coradofor histological analyses. A histological cut of each rat was obtained, and 10 photos of each blade were made per mouse with a 4x lens. As there were three rats per experiment, 30 photos were obtained per experiment.

The images were analyzed by the ImageJ software used for image processing and analysis, developed by Wayne Rasband at the National Institute of Mental Health, USA, in Java language. With this software, you can view, edit, analyze, process, save and print images. In ImageJ, the calculation of the areas is done by counting pixels of the regions selected by the user or by a specific algorithm [13]. ImageJ acts on the image by the intensity, or gray level of the pixels [14].

In the ImageJ software, an adjustment was made to allow the quantification of collagen in the histological slides. Measurements were made in µm2 and in percentage throughout the visual field of the image. The results are expressed in table 1 and figure 1.

Table 1. Description of areas in µm2 and percentages of collagen in histological sections of connective tissue of Winstar rats submitted to treatment with micro current, collagen, and DMAE. Gurupi, Tocantins, Brazil 2022.

		Control		***Collagen***		***DMAE***		***Micro current***	
		Area (μm^2)	*%*	*Area(μm^2)*	*%*	*Area (μm^2)*	*%*	*Area (μm^2)*	*%*
Mouse1 1	*Image 1*	*590,085*	*54,851*	*666,084*	*61,916*	*560,787*	*52,128*	*857,465*	*79,705*
	Image 2	*364,067*	*33,842*	*316,455*	*29,416*	*390,876*	*36,334*	*438,955*	*40,803*
	Image 3	*142,041*	*13,203*	*316,455*	*29,416*	*489,082*	*45,463*	*377,653*	*35,105*
	Image 4	*309,181*	*28,740*	*548,410*	*50,977*	*340,158*	*31,619*	*473,007*	*43,968*
	Image 5	*465,729*	*43,292*	*310,619*	*28,874*	*258,570*	*24,035*	*306,535*	*28,494*
	Image 6	*234,634*	*21,810*	*303,537*	*28,215*	*439,562*	*40,859*	*506,436*	*47,076*
	Image 7	*530,681*	*49,329*	*610,514*	*56,750*	*634,577*	*58,987*	*308,019*	*28,632*
	Image 8	*372,894*	*34,662*	*654,300*	*60,820*	*120,973*	*11,245*	*229,659*	*21,348*
	Image 9	*508,335*	*47,252*	*676,310*	*62,866*	*153,313*	*14,251*	*764,907*	*71,102*
	Image 10	*286,492*	*26,631*	*330,551*	*30,726*	*128,716*	*11,965*	*343,597*	*31,939*
Mouse222	*Image 1*	*286,019*	*26,587*	*251,094*	*23,340*	*139,857*	*13,000*	*258,940*	*24,070*
	Image 2	*476,142*	*44,260*	*455,458*	*42,337*	*363,149*	*33,756*	*653,715*	*60,766*
	Image 3	*349,382*	*32,477*	*255,377*	*23,739*	*517,003*	*48,058*	*174,672*	*16,237*
	Image 4	*335,151*	*31,154*	*627,965*	*58,372*	*758,865*	*70,540*	*174,672*	*16,237*
	Image 5	*516,840*	*48,043*	*700,333*	*65,099*	*276,614*	*25,713*	*210,345*	*19,553*
	Image 6	*645,405*	*59,993*	*706,777*	*65,698*	*275,490*	*25,608*	*162,003*	*15,059*
	Image 7	*652,277*	*60,632*	*420,049*	*39,046*	*665,124*	*61,826*	*548,059*	*50,945*
	Image 8	*236,181*	*21,954*	*258,359*	*24,016*	*291,649*	*27,110*	*525,446*	*48,843*
	Image 9	*522,940*	*48,610*	*330,503*	*30,722*	*800,528*	*74,413*	*540,222*	*50,216*
	Image 10	*599,100*	*55,689*	*248,837*	*23,131*	*622,652*	*57,878*	*671,964*	*62,462*
Mouse 3 3	*Image 1*	*383,018*	*35,603*	*451,144*	*41,936*	*154,031*	*14,318*	*364,821*	*33,912*
	Image 2	*509,539*	*47,364*	*390,352*	*36,285*	*314,655*	*29,249*	*271,387*	*25,227*

Image 3	*186,950*	*17,378*	*297,163*	*27,623*	*267,520*	*24,867*	*265,538*	*24,683*
Image 4	*589,892*	*54,833*	*212,007*	*19,707*	*316,989*	*29,466*	*188,744*	*17,545*
Image 5	*349,805*	*32,516*	*386,429*	*35,920*	*559,039*	*51,965*	*621,479*	*57,769*
Image 6	*218,691*	*20,328*	*550,538*	*51,175*	*405,768*	*37,718*	*155,869*	*14,489*
Image 7	*589,054*	*54,755*	*264,019*	*24,542*	*568,045*	*52,802*	*132,717*	*12,337*
Image 8	*149,173*	*13,866*	*249,260*	*23,170*	*561,544*	*52,198*	*538,047*	*50,014*
Image 9	*167,511*	*15,571*	*541,203*	*50,307*	*545,981*	*50,751*	*293,787*	*27,309*
Image 10	*153,924*	*14,308*	*142,317*	*13,229*	*327,503*	*30,443*	*251,471*	*23,375*

%: Percentage

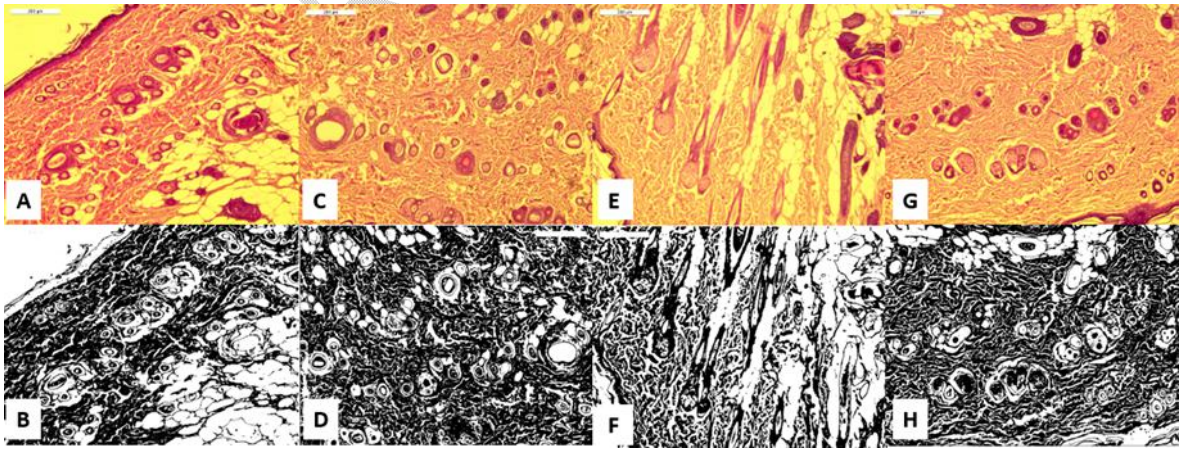
Table 2. Analysis of descriptive and statistical variables, by ANAVA test, of area measurements in µm2 and percentage of collagen in histological sections of connective tissue of Winstar rats submitted to treatment with micro current, collagen, and DMAE. Gurupi, Tocantins, Brazil 2022.

	Control		**Collagen**		**DMAE**		**Micro current**	
	Area (µm^2)	%	Area (µm^2)	%	Area (µm^2)	%	Area (µm^2)	%
Average	390,266	35,76	415,366	38,13	407,766	37,43	386,466	35,50
Standard deviation	163,044	15,12	169,783	15,71	190,954	17,66	197,249	18,26
CI 95%	144,5 a 285,5	24,57 a 46,96	252,64 a 323,85	26,80 a 49,31	236,96 a 236,96	25,90 a 48,82	152,40 a 274,32	23,77 a 46,82
P	-		0,0441		0,250		0,200	

IC: Confidence Interval; p: Significance level; %: Percentage

There was no difference between the DMAE and Micro Current group ~~with the~~.

Fig. 1.Images of histological sections of connective tissue, cordoned with HE (A: Control, C: Collagen, E: DMAE and G: Micro Current) and treated by ImageJ (Images B: Control, D: Collagen~~: ,~~: F: DMAE and H: Micro Current). Gurupi, Tocantins, Brazil 2022.



4. DISCUSSIONS

During the aging process, the amount of acetylcholine, elastic fibers, and collagen in the skin tissue gradually decreases. The most efficient way to obtain firmer skin and stronger muscles is by introducing and biostimulating rejuvenation precursors through aesthetic procedures [15].

However, the need to evaluate the effects of DMAE, Microcurrent, and oral collagen intake under the cutaneous tissue through histological blades effectively certifies the amount of collagen after the treatments proposed in this research.

In [16] conducted a study to evaluate the effects of hydrolyzed collagen on skin photoaging in rats. The authors observed through histological analysis that in the treatment group there was the repair of elastic fibers, and collagenous fibers, in addition to maintaining the proportion of collagen type II.

A recent study conducted by [17] sought to evaluate through a cutometer the effects of hydrolyzed collagen intake for four weeks on skin elasticity in postmenopausal women. The results found showed positive statistical significance compared to the placebo group. Although this study was conducted with humans, it was possible to observe through physical evaluation the effects of collagen on skin tissue.

Conducted a study [18] evaluating the effect of micro current on skin tissue repair in rats. Twenty-six animals were then used: 13 in a control group and 13 in intervention groups (manual passage of the current, lasting two minutes, for three weeks). In the histopathological analysis, there was no significant effect in relation to fibroblast proliferation, corroborating the results of this study compared with the other techniques studied.

Another study evaluated the effects of micro current in Wistas rats in which 12 animals were submitted to daily treatment for 10 days. After histological analysis, the authors observed an increase in angiogenesis, collagensynthesis and number and quality of fibroblasts presenting synergistic action on skin tissue compared to the control group [19]. This contradicts the results of this study, since there was convergence with the proposed treatment days.

Studied [20] the effects of DMAE on skin aging. The intervention consisted of the topical use of DMAE for 30 days in women, resulting in improved hydration, firmness, elasticity and skin viscosity. Although, this study presents as an intervention the use of injectable DMAE in rats, it was possible to notice agreement in the biomechanical effects of the skin, since the collagen network positively induces these characteristics.

4. CONCLUSION

Currently, several resources are available for the prevention of skin aging and to induce collagen stimulation is to delay this event. In this study, the use of oral collagen for seven days in animals proved to be efficient after histological analysis, which is in contact with the bibliography presented. Therefore, this stimulus, associated with the other interventions of this study may be predictors for a more efficient result, since all interventions were effective in the collagen network. However, further studies with this methodology should be carried out in order to observe this effect on human subcutaneous tissue.

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