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An autologous anti-aging serum confirms its beauty enhancer effect but its role as a chronic inflammation modulator is not clear

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Abstract: There are many biological theories that claim to be the final explanation of aging, though actually it is well accepted that none of them provides an explanation that allows a full understanding of the complicated, multi-factorial, unavoidable and deleterious aging process. Inflammation can be considered a core process of aging and vice versa, aging is sometimes referred as a chronic inflammatory state condition. AAS is highly concentrated in some growth factors and anti-inflammatory cytokines like Interleukin-1 receptor antagonist (IL-1ra). Evidence suggested that AAS had two very well differentiated clinical effects: a systemic "anti-inflammatory-anti-aging" action and a local aesthetic action. To confirm the two effects with a multisession protocol and to assess a possible correlation between them were the aims of this work.

Keywords: Aging, Autologous Antiaging Serum (AAS), Beauty, Interleukin-6, C Reactive Protein, Chronic Inflammation

1. Introduction

There are many biological theories that claim to be the final explanation of aging, though actually it is well accepted that none of them provides an explanation [1] that allows a full understanding of the complicated, multi-factorial, unavoidable and deleterious aging process. During the last decade, the "Inflammation Theory of Aging" (sometimes considered as a sub-theory [2] of the "Free Radical Theory of Aging [3]") has been gaining adepts. Briefly, it states that constantly increasing pro-inflammatory cytokines are a consequence of bystander damage and the cause of further cell deterioration (aging).

Inflammation can be considered a core process of aging [4] and vice versa, aging is sometimes referred as a chronic inflammatory state condition. Evidence has shown significant correlation between the aging process and: chronic inflammation [4] and/or diseases with a subjacent chronic inflammatory state (age-related diseases) [5]. AAS is highly concentrated in some growth factors and anti-inflammatory cytokines like Interleukin-1 receptor antagonist (IL-1ra), as detailed in some of our previous works [6].

The initially gathered data suggested two very well differentiated clinical effects of AAS: a systemic "anti-inflammatory-anti-aging" action (lowering of chronic inflammation markers associated to aging, degenerative diseases and/or dysfunctional parameters) and a local aesthetic action, improving cell metabolism and tissue beauty properties. In previous pilot studies, we witnessed these AAS anti-aging [6] and aesthetic effects [7, 8], though they were never assessed simultaneously. To confirm the two effects with a multisession protocol and to assess a possible correlation between them were the aims of this work.

2. Materials & Methods

Subjects. Inclusion criteria: a) 30 to 60 years old women; b) no systemic pathologies; c) no record of cancer or malign lesions; d) not undergoing any chronic treatment or receiving any daily medication; f) had not undergone aesthetic treatments or procedures from a month prior to first session to a month after last one; g) had a blood analysis performed 15 days or less before the first therapeutic session; h) had not suffered a body weight fluctuation of 3% or above; i) not pregnant or breastfeeding.

Samples. Every subject had measurements recorded 24 hours prior to the 1st therapeutic session (control

measurements, S0) and 45 days after the 4th therapeutic session (S1). Figure 1.

Device. It contains highly purified special borosilicate medical grade glass spheres that stimulate the cells to produce growth factors and anti-inflammatory components without increasing significantly the pro-inflammatory factors at the same time [9].

Measurements. Data was obtained in the same surgery theatre where treatments were performed, under controlled ambient conditions and using different test probes:

a) Cutometry. By applying suction, the skin gets absorbed into a hole in a probe. The amount of skin that enters the hole is directly related to elasticity and other mechanical properties of the skin and can be accurately measured. The variables registered were: R0, passive behavior of the skin to force; R2, skin gross elasticity; R5, skin net elasticity; R6, viscoelastic component on the elastic part of the curve; R9: tiring effects of the skin after repetitive stimuli; F0, theoretical value (elasticity); F4, theoretical value (skin firmness); Q0, maximum recovery area, (firmness). Parameters: 450 mbar pressure, 2 mm diameter probe hole, 2 seconds suction time, 10 repetitions, 2 seconds in between suction time. Cutometer® MPA 580, Courage Khazaka, GMbH, Germany.

b) Corneometry. A capacitive electric current is applied to the skin. The resistance it finds in its way back to the probe is in accordance to epidermal hydration. This variable is registered as a %) Parameters: 7 measurements, 1 second time for each measurement, frequency: 0.9-1.2 MHz. Corneometer® CM825, Courage Khazaka, GMbH, Germany.

c) Frictiometry. A disc in a probe spins in contact with the skin. The resistance the tissue offers against this movement can be measured. A validated scale provided by the manufacturer (from 1 to 1000 pts) was used. Parameters: 16 mm Teflon head, 0,7 N pressure, 30 seconds record time, 45º angle measurement. Frictiometer® FR700, Courage Khazaka, GMbH, Germany.

d) IL-6. ELISA Immunoassay (0,005 pg/mL limit of detection).

e) usCRP. Turbidimetric quantification (0,05 mg/L limit of detection).

Therapeutic session. The sample processing involved 5 sequential steps: blood extraction, blood incubation, AAS preparation, AAS storage and AAS application.

1. Blood Extraction: standard extraction procedure. Left arm venous blood was obtained using a Vacutainer™ system. 30 ml were collected in the special tube/syringe device. Blood was collected in five fully filled devices at every extraction.
2. Blood Incubation. The 5 blood filled devices were incubated at 37ºC for 24 hours. During this time, the blood remained exposed to 30 2mm borosilicate medical grade glass pebbles inside each tube.
3. AAS preparation. The 5 devices underwent a centrifugation protocol. Centrifugation was performed in a single cycle of 10 minutes at 5000 rpms. With this centrifugation protocol the samples were exposed to 3000g. Once the 5 devices had been centrifuged and rested in a test tube rack, the AAS was extracted individually from each device. Standard 5 ml Luer Lock™ syringes with 20G x ¾ needles were used. Approximately 3,6 ml of AAS were obtained from each device.

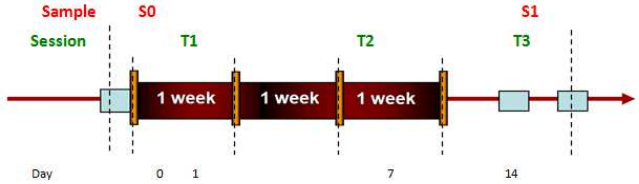

Figure 1. *Therapeutic sessions (T1, T2, T3 and T4) and sample obtaining (S0 and S1) schedule.*

4. AAS Storage. Out of the four 5 ml syringes with the AAS obtained (above), two were stored. For this purpose a cap was attached to these two syringes and both were kept frozen (-20ºC) after proper labelling. The stored syringes were kept in these conditions for 15 days until they were taken out, 40 minutes before the second session application time.
5. AAS Application. An application session consisted of several low-volume intra-dermal (ID) injections –mesotherapy-. These were performed manually using a standard mesotherapy technique (leaving no papulae) with a 30G ½/10 mm needles and attaching a 0,2 µm filter to the syringe. One vial (3,6 – 3,8 ml AAS) was injected at a depth of 3 mm along the application zone: 1,8 - 1,9 ml in the right malar region and 1,8- 1,9 ml in the left malar region. Every application lasted approximately 15 minutes. Alcohol 70º was the chosen antiseptic agent.

Traceability. Every sample was carefully labelled and stored. To assure the correct traceability and guaranty proper AAS handling, labels and special carton racks were used. All syringes were carefully identified at all times. When stored or incubated, all AAS syringes belonging to the same patient were put in a specific single use labelled and sealed carton rack.

Analysis. For every variable analysed a "Shapiro-Wilk Test" was used to assess normal-distribution. When normal-distribution was verified, a "Student T Test" was used to compare means. When normal-distribution was rejected, non-parametrical "Mann-Whitney U Test" was used. SPSS 17.0 for Windows® (Statistical Product and Service Solutions Ibérica, S.L.U., Madrid, España) was the software used for statistical analysis.

3. Results

The analysed sample consisted of 30 women recruited consecutively.

a) Cutometry (n=30): Table 1.
b) Corneometry (n=26). S0 mean hydration 46,095% (SD 7,844) and S1 mean hydration 55,722% (SD 8,999); there is a 9,63% (CI 95% 4,93 to 14,33) hydration enhancement; p < 0,001.
c) Frictiometry (n=27). S0 mean smoothness 469,24 pts (SD 172,446), S1 mean smoothness 356,61 pts (SD 167,117). There is a mean difference of 112,63 pts (IC 95% 16,81 to 208,45); p = 0,022.
d) IL-6 (n=30): Figure 2. S0 mean concentration: 0,125 pg/ml (SD: 0,56), S1 mean concentration: 1,146 pg/ml (SD: 2,481). p<0,001.
e) CRP (n=30): Figure 3. S0 mean concentration: 0,1481 mg/dl (SD: 0,141), S1 mean concentration: 0,442 mg/dl (SD: 0,341). P=0,001.

Table 1. *Cutometry variables comparison before and after ACAS treatment.* [a] *ST: Standard Deviation;* [b] *CI 95%: confidence interval 95%;* [c]*p: statistical significance;* [d] *U Mann-Whitney Test;* [e] *t Student Test.*

Cutometry	Pre AAS (ST[a])	Post AAS (ST[a])	Post AAS (ST[a])	Diff. (CI 95 [b])	p [c]
R0	0,288 (0,057)	0,222 (0,043)	0,222 (0,043)	0,666 (-)	<0,001[d]
R2	0,714 (0,112)	0,722 (0,089)	0,722 (0,089)	-0,008 (-0,065 to 0,049)	0,772[e]
R5	0,451 (0,116)	0,554 (0,136)	0,554 (0,136)	0,010 (0,033 to 0,172)	0,005[e]
R9	0,075 (0,164)	0,057 (0,015)	0,057 (0,015)	0,018 (-)	<0,001[d]
F0	9,723 (2,037)	7,507 (1,441)	7,507 (1,441)	2,216 (-)	<0,001[d]
F4	13,365 (2,472)	12,676 (2,803)	12,676 (2,803)	0,689 (-0,677 to 2,055)	0,317[e]
Q0	57,12(11,202)	46,06 (10,569)	46,06 (10,569)	11,06 (-)	0,001[d]

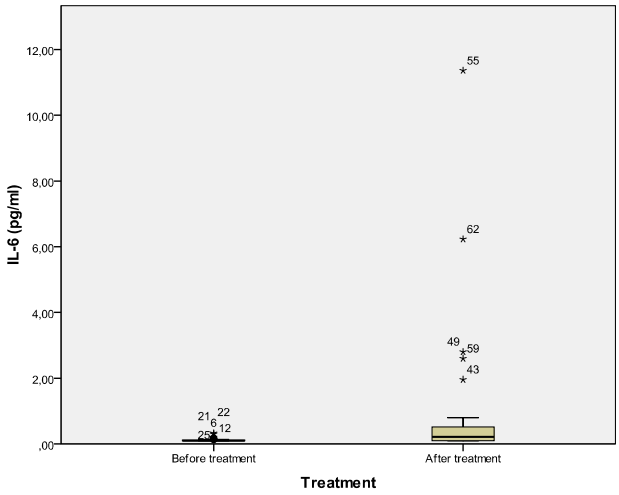

Figure 2. *IL-6 pre and post treatment blood concentrations (pg/ml).*

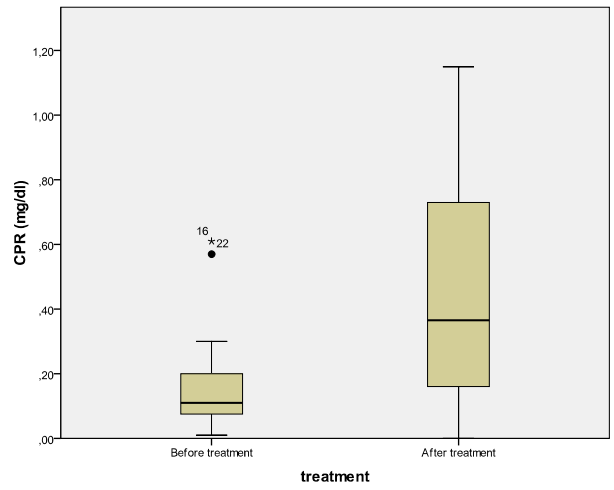

Figure 3. *CRP pre and post treatment blood concentrations (mg/dl).*

4. Discussion

In our last study, we obtained results that suggested an improvement of skin beauty (hydration 17,08%, firmness 10,38% and viscoelasticity 16,59%) by applying 2 AAS vials (1 vial: face mesotherpay, 1 vial: gluteus intramuscular) in 2 sessions separated by 15 days. In a series of 8 cases, we also recorded a 94,52% IL-6 blood concentration reduction and a 13,27% CRP blood concentration reduction by applying 2 intramuscular vials in 2 sessions separated by 15 days. The follow-up time was 45 days in both studies. While the aesthetic improvements of the skin of the face were a consequence of a direct AAS local action, chronic inflammation markers reduction seemed to be independent from the AAS administration via.

In this new study, the AAS final dose was the same that was used in the previous studies (4 vials per month), but the protocol suffered changes: i) all vials were injected intra-dermis, ii) only 1 vial was injected per session, iii) weekly sessions and iv) extended follow-up time to 65 days. The idea was to be able to obtain a similar impact on chronic inflammation markers with even better results for skin beauty improvement, as a previous step to a future larger dose application study. But some results were consistent with our previous findings and some other were not.

a) Cutometry. Proper assessment of the mechanical properties of the skin is a very complicated matter. There are many tissues and forces interacting together and since the cutometer recovers data from up to 18 different variables with every measurement, there are always incoherent or at least not consistent parameter values. This is the case of R0 and R2. In previous studies we registered a 10,38% increment but in this study a R0 reduction was observed instead. The clinical consequences of such a reduction suggest that these figures cannot be real. Further studies should provide an explanation to this incoherent

finding. R2, on the other hand, showed a 1,12% increment, not consistent but significantly different to the 16,59% increment found before. The opposite occurred with R5, that showed a 22,84% increment, 2-fold the 11,21% registered previously, reinforcing the idea of a much more elastic condition of the skin. As with R5, R9 showed a very important change: a 31% reduction. This stands for a much better response to repetitive stimuli, a less tiring effect.

F0 is an area deducted from the total area of the cutometry curve. A 22,79% reduction reflected an important improvement of the elasticity of the skin that does not correlates with the almost irrelevant improvement showed by R2. The F4 parameter is also an area and it accounts for skin firmness. A reduction of 5,16% implies a more firm skin and is consistent with the 10,38% improvement found in previous studies. Q0 shows the maximum recovery area of the cutometry curve, which is related to the skin firmness. The 19,36% reduction observed in this parameter is a very important figure that not only backs-up the results registered in previous studies, but also reinforces the idea of the biased R0 data mentioned above.

b) b) Hydration: the 9,63% reduction recorded in this study was consistent with 17,08% previously recorded. The smaller increment registered may be justified in the extended follow-up time, suggesting that when AAS is applied in this fashion, a single vial action may last no more than two months. Further studies should clarify this.

c) Smoothness: a 24% improvement was registered in this study. This was important and unprecedented data for in previous studies this variable was biased. Smoothness is a qualitative parameter of beauty and its improvement should find correlation with other similar parameters. Further measurements of qualitative parameters are required to assess this beauty component properly.

d) Blood markers: IL-6 and CRP. In previous studies we registered the following data: n=8, IL-6 mean pre-treatment blood concentration 1,150 (SD 0,272); IL-6 mean post-treatment blood concentration 0,063 (SD 0,042), p=0,002 and CRP mean pre-treatment concentration 0,113 (SD 0,005); CRP mean post-treatment concentration 0,098 (SD 0,019), p=0,426. In this study, results are contradictory. Not only reductions in the IL-6 and CRP blood concentrations were not achieved, but they raised-up notoriously. Though it was a small sample with an important dispersion, these results were not expected. The mean IL-6 blood concentration was dramatically affected in particular by two extreme outlier values. The most extreme outlier value subject was lost to follow up. The second extreme outlier value did not develop any pathology or infection in the days that followed the treatment that may justify this concentrations. In this study, the serum was administered on a weekly basis instead of sessions separated by 15-days. Thus, in comparison to our previous studies, local damage due to facial mesotherapy injections was doubled up. IL-6 and CRP blood concentration increments are highly unspecific. Further investigations should back-up or dismiss these findings and provide a correlation between AAS injection and blood markers.

e) Number of sessions and administration via. Results are controversial. It is not clear whether the results achieved with four weekly one-vial sessions are better than the ones achieved when injecting two vials every 15-days. The same happens when comparing intra-dermis and intra-muscular via. Since needle prick itself has beneficial effects on beauty, it is logical to assume that the more intra-dermis injected vials, the better the results. On the other hand: could the higher number of intra-dermis injections be behind the CRP concentrations increment? Future research should provide the answer to this question.

AAS role as a beauty enhancement treatment becomes clearer with every new study: hydration, smoothness and several mechanical properties of the skin are clearly improved by the injection of this serum. Previous publications are confirmed by this work. Still, there are some considerations that must be stated and should be the basis of AAS future research: a) cutometry showed some inconsistent data, b) clinical improvements seemed to diminish around the second month after the application, suggesting identification a possible limit of the duration of the beauty-enhancing effect, c) the anti-aging effect is still uncertain, since chronic inflammatory markers responded differently in several studies, d) results can only be extrapolated to female population.

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