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Modulation Possibilities of the Tissue Wettability, Marker of the Physical Modifications Produced by Senescence versus Photo Senescence at Tegument Level

Laurentiu Badescu[1], Carmen Elena Cotrutz[1], Raoul Lupusoru[2], Bogdan-Mihail Diaconescu[2], Manuela Ciocoiu[2*], Magda Badescu[2]

[1]Department of Cell and Molecular Biology, University of Medicine and Pharmacy "Grigore T. Popa", Iasi, Romania
[2]Department of Pathophysiology, University of Medicine and Pharmacy "Grigore T. Popa", Iasi, Romania
Email: [*]mciocoiu2003@yahoo.com

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Abstract

We used an experimental model involving white Westar rats (young and old) who were subjected to precise doses of ultraviolet radiation (UV). The experiment aims to examine the influence of epidermis potential protection factors in photo senescence versus senescence. We have studied the *in vivo* effect of some natural polyphones extracted from black grapes seeds and zinc aspartate and *in vitro* effect of AED (deuterium depleted water). The substances were administered by intragastric gavages, at two days intervals for two weeks prior to UV irradiation and during the entire period of the experiment (28 days). In the end, we determined the degree of hydrophilicity of the skin, *in vitro*, by measuring the contact angle value, which was inversely proportional to the hydrophilicity of the tissue (the angle formed at the contact between a liquid and a solid surface). Using this method we found a large variability depending on the hydrophilicity of the epidermis (abdomen and back), the age of the animal and UV/non UV irradiation. The maintenance of the epidermis fragments in AED (for 6 hours) increased significantly the tissue wet ability degree both in the young rats subject to UV and in the senescent ones subject to UV (photo senescence).

Keywords

Skin, Photoimmunology, Phototherapy

[*]Corresponding author.

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1. Introduction

Chronic exposure to sunlight can cause premature aging of the skin and in some cases can promote tumor growth. Aging caused by ultraviolet radiation (UV) exposure can manifest into two ways: immediate (short term) tanning with possible sunburns and late changes such as skin thickening, decreased skin elasticity and firmness loss [1]. Sometimes prolonged exposure can lead to precancerous lesions or even cancer. The main body protection against solar radiation is the presence of melanin in melanocytes. Changes detected on skin biopsy revealed complex alterations on keratinocyte differentiation and kinetic and structural components of the skin in the context of photodamage [2] overlapped to the physiological senescence. The extracellular matrix, the main location of chemicals exchange loses hyaluronic acid with age and becomes less permeable, less hydrated and less fluid [3].

We determined the degree of hydrophilicity of the skin, *in vitro*, by measuring the contact angle value, which is inversely proportional to the hydrophilicity of the tissue.

The experiment aims to examine the influence of potential epidermis protection factors in photo senescence versus senescence [4]. So we studied the *in vivo* effect of some natural polyphenols and zinc aspartate [5] and *in vitro* effect of AED (deuterium depleted water). We used a UV lamp produced by INMB (National Institute of Metrology Bucharest). The lamp was placed at a distance of 20 - 30 cm from the animal and the dose was 2.4 J/cm^2/day for 28 days.

2. Materials and Methods

This desideratum was carried out by determining the tissue wettability degree of the tegument [6]. The contact angle is the angle formed at the contact area between a liquid and a solid surface. As previously mentioned, the q contact angle value depends on the surface roughness.

Therefore, if the liquid drop is deposited on a rough surface, one can notice two distinct situations, namely: a) the Wenzel regimen, where the water penetrates completely through the asperities—the case of the epidermis; b) the Cassie-Baxter regimen, where the drop remains hung up on the asperities apices (fakir drop) (**Figure 1**).

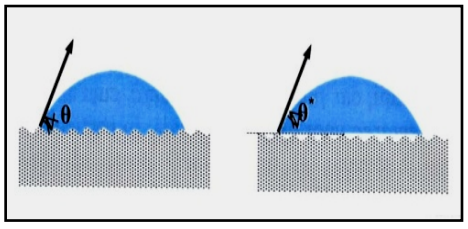
The best assessment of the contact angle is obtained through the numerical solving of the differential equation of the profile of the drop dispersed on the surface, and the calculation error depends on the numerical error adopted and on the errors regarding the experimental measuring of the main dimensions of the drop, y and x (**Figure 2**).

Moreover, the drop volume has to be small enough, so as to neglect its mass. Consequently, we have always worked with the same automatic micropipette. Usually, assessment errors of ±2˚ of the contact angle are accepted. The measurement device of the contact anglesis called goniometer (**Figure 3**) and it was designed by Zisman in 1960.

As a consequence, by experimentally determining the t angle and knowing the superficial pressure of the liquid and the solid-vapors interfacial energy, one can determine the solid-liquid interfacial energy, namely the practical expression of the wet ability degree of the solid (skin) [7].

By using this method, we have noticed a great variability of the epidermal wet ability, according to the tegument area (abdomen or back), to the age of the experience animal and to the UV irradiation UV or non irradiation [8].

We sampled tegument fragments in the flanks area, from animals having different ages which were subject or not to the UV rays, for 4 weeks.



(a) (b)

Figure 1. (a) The liquid drop on the rough surface, in the Wenzel model; (b) Cassie-Baxter regimen (N. Dumitrascu-"Biomaterials and Biocompatibility", 2007).

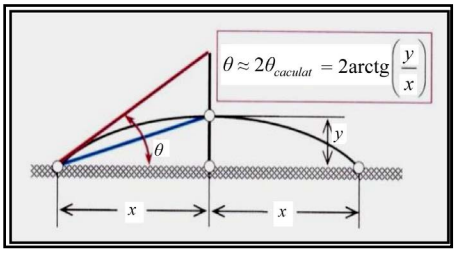


Figure 2. The calculation of the contact angle on the plane surface.

Figure 3. The goniometer employed by us (Tropfenkontur-Analyse system DSA100).

Then, we separated the epidermis from the dermis and we exposed it on a blade. Then, we read on the goniometer the q value. We introduced the fragments from the homologous regions in water poor in deuterium for 6 hours and we repeated the readings on the goniometer.

3. Results and Discussion

The contact angle values differ significantly from a statistical point of view, both among the groups of young witnesses and among the groups of young rats subject to the UV. For the groups of young rats, the value ranges of the contact angle through the variability coefficient indicates the homogeneity of the groups of young rats subject to the UV radiations, as compared to the corresponding young control groups.

The contact angle assessment in the young rats in diverse experimental conditions highlight the following aspects [9]: the probability that the two value ranges of the contact angle, obtained in the young control groups and young rats subject to UV radiations, will not differ is very small ($F = 2.67 \times 10^{-6}$) and moreover, between the young control group + AED and the group of young rats subject to UV radiations + AED, the probability that the two value ranges of the contact angle will not differ was very small ($F = 4.80 \times 10^{-9}$).

The small values of the F test are confirmed, by carrying out the t-Student test, the value ranges of the contact angle in the young control group and in the rats subject to the UV in different experimental conditions highlight strong statistical differences (**Table 1**, **Figure 4**).

The contact angle values differ significantly from a statistical point of view, both among the senescent control group and among the groups of senescent rats subject to the UV radiations. For the groups of senescent rats, the ranges of values of the contact angle through the variability coefficient indicate the homogeneity of the control groups.

The contact angle assessment in the senescent rats in diverse experimental conditions highlights (**Figure 5**): —the probability that the two value ranges of the contact angle, obtained in the senescent control groups and senescent rats subject to UV radiations, will not differ is of 26%;—between the senescent control group + AED

Table 1. The statistical differences of the average values of the contact angle in the young rats, on study groups.

Lot (GL = 22)	Mt + AED	Lot (GL = 22)	UVt + AED
Mt	(43, 15) $p < 0.001$	UVt	(10, 33) $p < 0.001$

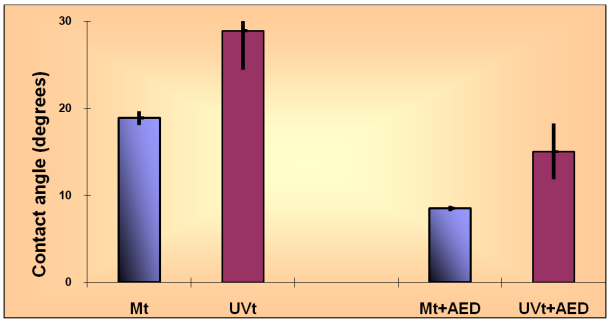


Figure 4. The average values of the contact angle in the young rats.

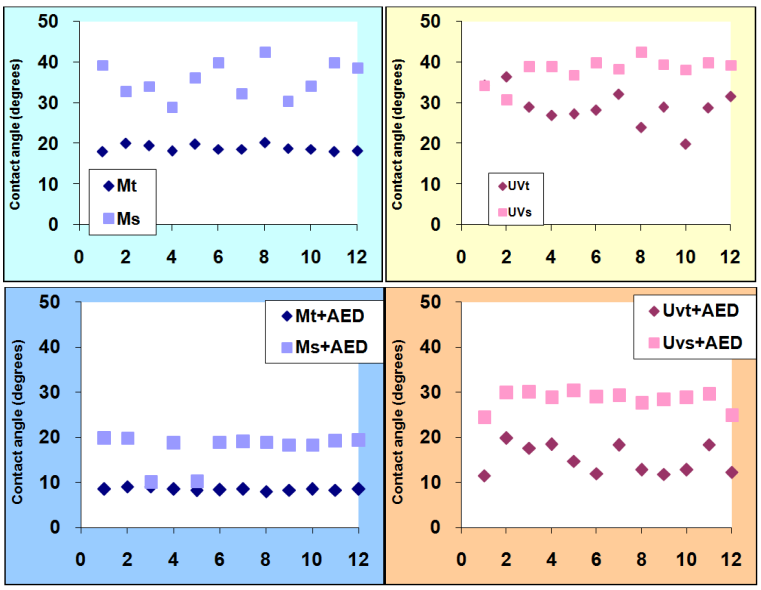


Figure 5. The average values of the contact angle in the senescent rats in diverse experimental conditions.

and the group of senescent rats subject to the UV radiations + AED, the probability that the two value ranges of the contact angle will not differ was of 6%.

By carrying out the t-Student test for the value ranges of the contact angle in the senescent control group and the rats subject to the UV radiations in diverse experimental conditions, one can notice significant statistical differences exceeding the unanimously accepted significance threshold of 95%.

The correlation between the contact angle values registered in the groups of young and senescent rats, according to the experimental conditions, shows strong significant statistical differences in all the cases. The wet ability is inversely proportional to the contact angle value.

One can notice a highly increased wet ability in the young groups, as compared to the senescent rats. The irradiation decreases significantly the wettability, regardless of the age. This is irreversible in the irradiated senescent groups and subject to AED and significantly reversible in the young groups subject to UV and treated with AED [10].

The AED effects of amelioration of the cutaneous cosmetological status are carried out through:

- the improvement of water balance performance (being a hypotonic water, it is absorbed much more quickly and in larger quantities within the cells) exercising an excellent hydration effect;
- the improvement of the use of the glucose and fats revitalizing the energetic and constitutional cutaneous cellular metabolism;
- the control of the cellular proliferation, with consequences on the epidermis, where it influences the cellular regeneration, on the dermis, where it modulates the collagen and conjunctive-elastic fibers synthesis [7].

By comparing the average values of the contact angle with the average values of the catalase or of the superoxiddismutase [11] in the groups of untreated control group of rats (**Table 2**), we can draw the following conclusions:—there are strong statistic differences both in the young rats and in the senescent ones;—the most significant differences are met for the catalase *vs* contact angle in the young rats, but also in the senescent ones.

Moreover, the control groups of treated rats present values of the t-Student test exceeding the significance threshold of 99%, the differences between the average values of the contact angle as compared to the Superoxide Dismutase free radical inhibitor (SOD), but most of all as compared to the catalaseand treated with zinc [12]-[15] are strongly significant from a statistical point of view.

By comparing the average values of the contact angle with the average values of the catalaseor of the superoxiddismutase, in the groups of untreated rats subject toultraviolet radiations (**Table 3**), we can draw the following conclusions:—there are strong statistical differences both in the young rats and in the senescent ones;—the most significant differences are met for the catalase *vs* contact angle in the senescent rats, but also in the young ones (**Table 4**, **Figure 6**).

The groups of treated rats subject to ultraviolet radiations present the following differences:—the t-Student test highlights strong significant differences between the average values of the contact angle as compared to the catalase, more significant in the groups of animals treated with zinc, both in the senescent animals, but mainly in the young ones;—between the contact angle and the SOD in the young animals there are no differences, and for the senescent animals these differences are strongly significant from a statistical point of view, with close values for the animals treated with polyphenolsor with zinc.

Table 2. Statistical differences of the average values of the contact angle, as compared to the catalase or superoxid dismutase in the groups of untreated control group of rats.

Contact angle differences	Young rats		Senescent rats	
	t-student	p	t-student	p
Catalase (GL = 22)	1050,88	$p < 0.001$	82.30	$p < 0.001$
SOD (GL = 22)	4.73	$p < 0.001$	10.12	$p < 0.001$

Table 3. Statistical differences of the average values of the contact angle, as compared to the catalase or to the SOD in the groups of untreated rats subject to UV radiations.

Contact angle differences	Young rats subject to UV		Senescent rats subject to UV	
	t-student	p	t-student	p
Catalase (GL = 22)	64.07	$p < 0.001$	193.56	$p < 0.001$
SOD (GL = 22)	12.87	$p < 0.001$	21.77	$p < 0.001$

Table 4. Statistical differences of the average values of the contact angle as compared to the catalase or to the superoxid dismutase.

Contact angle differences	Young rats		Senescent rats	
	t-student	p	t-student	p
Rats treated with polyphenols				
Catalase (GL = 22)	38.55	$p < 0.001$	49.86	$p < 0.001$
SOD (GL = 22)	0.29	NS	13.54	$p < 0.001$
Rats treated with zinc				
Catalase (GL = 22)	94.15	$p < 0.001$	50.35	$p < 0.001$
SOD (GL = 22)	1.90	NS	12.31	$p < 0.001$

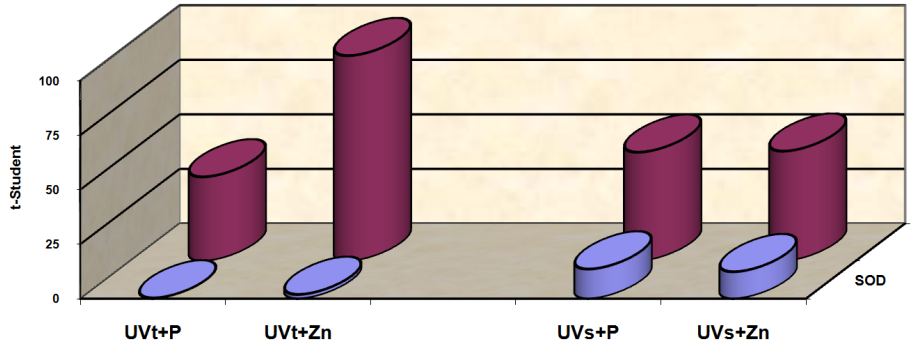


Figure 6. Statistical differences of the average values of the contact angle as compared to the catalase or to the superoxid dismutase.

4. Conclusions

We have determined for the first time the wet ability degree of the tegument, *in vitro*, by measuring the contact angle value, inversely proportional to the tissue wet ability. We have proved that the UV rays irradiation decreases significantly the wet ability of the epidermis, regardless of the age. One can notice a highly increased wet ability in the young groups, as compared to the senescent rats.

The irradiation decreases significantly the wet ability, regardless of the age. This is irreversible in the irradiated senescent groups and subjects to AED and significantly reversible in the young groups subject to UV and treated with AED. By comparing the average values of the contact angle with the average values of the catalaseor of the superoxiddismutase, in the groups of untreated control group of rats, we can draw the following conclusions: there are strong statistic differences both in the young rats and in the senescent ones; the most significant differences are met for the catalase *vs* contact angle in the young rats, but also in the senescent ones. The groups of treated rats subject to ultraviolet radiations present the following differences: the t-Student test highlights strong significant differences between the average values of the contact angle as compared to the catalase, more significant in the groups of animals treated with zinc, both in the senescent animals, but mainly in the young ones; between the contact angle and the SOD in the young animals there are no differences, and for the senescent animals these differences are strongly significant from a statistical point of view, with close values for the animals treated with polyphenolsor with zinc.

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