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Evaluation of the human skin responses to solar-simulated radiation in an *ex vivo* model: effects and photoprotection

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Introduction

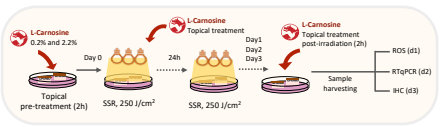
Terrestrial solar radiation is one of the most important environmental factors affecting skin physiology [1]. Its spectrum could be divided into three main wavelength portions: ultraviolet (UV, 5 %), visible (VIS, 50 %), and infrared (IR, 45 %). The exposure of human skin to sunlight, and more specifically the UV component, can lead to short- and long-term consequences including erythema, photo-aging, photo-immunosuppression and skin cancers [2-5]. For this reason, it is important to study the impact of solar exposure on the skin and develop new photoprotective compounds to avoid or reduce its damaging consequences.

The purpose of this work is to set up a methodology that could be used to verify the effects of solar radiation on human skin and to assess the capacity of different compounds in protecting the irradiated skin. *Ex vivo* human skin exposed to solar-simulated radiation was used as model to investigate oxidative stress induction, inflammatory response, and photo-aging by evaluating ROS formation, transcriptional response, and proteins level. Specifically, *ex vivo* skin exposed to solar-simulated radiation (SSR) was used as a model to test L-Carnosine as photo-protective treatment.

Materials & Methods

SSR SOURCE	MODEL	READOUTS
ATLAS SUNTEST XLS+ ~ 300-800 nm	*Ex vivo* human skin (biopsies) Air-liquid interface culture Daily exposure to SSR (250 J/cm²)	ROS induction (day 1) Gene expression (day 2) Proteins level (day 3)

STUDY DESIGN



Conclusions

- Our SSR model on ex vivo human skin is a valuable system to assess the consequences of solar light and the capacity of applied compounds to counteract them.

- L-Carnosine treatments decrease SSR induced ROS production.

- L-Carnosine helps to counteract the effect of SSR on genes involved in inflammation (**IL20, IL6, PTGS2, TNF**), extracellular matrix remodelling (**CYR61**), and other biological functions (**FGF7, GDF15**)

- L-Carnosine helps to reduce PTGS2 and CYR61 proteins level, respectively involved in skin inflammation and skin connective tissue aging.

Results & Discussion

L-Carnosine promotes a reduction of ROS production induced by SSR

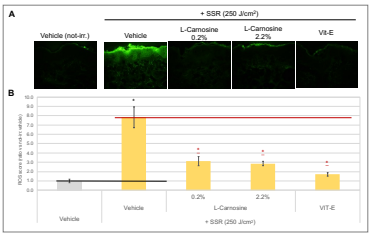


Fig. 1. Decrease of ROS production in L-Carnosine treated samples. Skin samples were treated 16h with vehicle or test compounds and stimulated with 250 J/cm² of SSR. ROS production was assessed by DCFH-DA assay and fluorescence obtained was evaluated in the upper dermis. A) Representative images. B) Graph showing ROS mean level (expressed as ratio vs the not-irradiated vehicle) obtained in two independent studies (n=2). Error bars indicate the standard error of mean (SEM). * Significantly different from not-irradiated vehicle, * Significantly different from irradiated vehicle (Tukey's test, p<0.05).

L-Carnosine helps to counteract the effect of SSR on gene expression

A

Target	Expected modulation after SSR	Fold-change (vs Not-irr. VEHICLE)								
		Don_1			Don_2			Don_3		
		+ SSR (250 J/cm2)			+ SSR (250 J/cm2)			+ SSR (250 J/cm2)		
		L-Carnosine			L-Carnosine			L-Carnosine		
		VEHICLE	0.2%	2.2%	VEHICLE	0.2%	2.2%	VEHICLE	0.2%	2.2%
GRIP1	-	0.3	0.1	0.2	0.2	0.1	0.2	0.5	0.4	**0.7**
PRKCB	-	0.3	0.1	0.2	0.0	0.1	**0.2**	0.4	0.4	0.3
IL20	+	8.2	**4.1**	**4.6**	14.7	**12.8**	**5.7**	1.5	**0.8**	**0.9**
IL6	+	4.2	**1.4**	**2.4**	13.3	**9.4**	**10.4**	2.6	2.2	**2.1**
TNF	+	12.9	**8.2**	**7.3**	6.6	7.7	**4.7**	1.4	1.4	1.2
OCA2	-	0.5	0.2	0.3	0.7	0.4	0.4	0.3	0.3	0.3
PTGS2	+	5.3	**4.2**	**4.0**	13.9	**11.3**	**7.7**	2.0	**1.4**	**1.4**
CYR61	+	8.1	**2.7**	**4.0**	8.1	8.2	**4.6**	2.3	2.9	**1.5**
FOSL1	+	4.8	4.6	4.5	11.2	**8.6**	**5.7**	2.2	3.1	4.8
FGF7	+	14.0	**1.0**	**5.2**	1.8	2.0	**1.0**	0.7	4.2	1.0
GDF15	+	67.4	**22.0**	**22.4**	16.5	**11.9**	**14.1**	57.3	**25.2**	**26.8**

B

L-Carnosine photo-protective effect			
Target	Don_1	Don_2	Don_3
GRIP1			✓
PRKCB		✓	
IL20	✓	✓	✓
IL6	✓	✓	✓
TNF	✓	✓	
OCA2			
PTGS2	✓	✓	✓
CYR61	✓	✓	✓
FOSL1		✓	
FGF7	✓	✓	
GDF15	✓	✓	✓

C

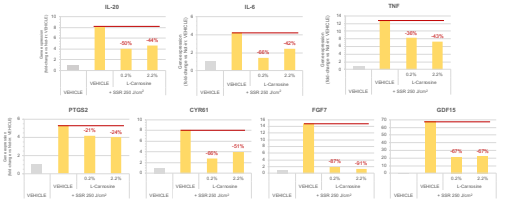


Fig. 2. L-Carnosine mitigates the gene expression modulation. Gene expression of the 11 selected genes was evaluated in skin biopsies from 3 donors treated with L-Carnosine 0.2% or 2.2% and exposed to SSR up to day 2. L-Carnosine treatment protects from the effect of SSR reducing the gene expression modulation of several genes. A) Gene fold-change values obtained in relation to the not-irr.vehicle (fold-change = 1.0). The expected gene modulation following SSR is highlighted in *grey* (fold change up-reg. ≥2 and down-reg. ≤0.5); in ***bold italics*** the fold-change values mitigated by L-Carnosine treatment (photo-protective effect, Δfold-change ≥0.5). B) Summary of the gene expression results; ✓ indicates L-Carnosine photo-protective effect at least with one concentration tested, and in *grey* if this effect is confirmed at least 2/3 donors. C) L-Carnosine reduces the effect of SSR on gene expression of 7/11 genes. Example of results obtained (Don_1).

After SSR, PTGS2 and CYR61 protein induction is mitigated by L-Carnosine

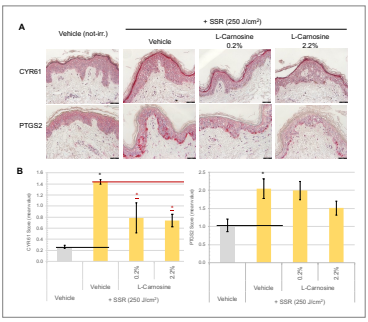


Fig. 3. L-Carnosine mitigates PTGS2 and CYR61 protein induction. PTGS2 and CYR61 protein level was evaluated in skin biopsies treated with L-Carnosine 0.2% or 2.2% and exposed to SSR up to day 3. Protein level was assessed by immunohistochemical staining. L-Carnosine treatment protects from the effect of SSR reducing the protein induction of both markers. A) Representative images. B) Graphs showing CYR61 and PTGS2 level (mean score). Error bars indicate the standard error of mean (SEM). * Significantly different from not-irradiated control vehicle; ▪ Significantly different from irradiated vehicle (Tukey's test, p<0.05).

- **L-Carnosine helps to mitigate ROS production and to counteract the modulation of genes involved in inflammation, photo-aging and stress response.**
- **L-Carnosine could be used in addition to UV-filter to provide better prevention against solar radiation.**

References

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