Pandemic stress and the role of sustained cortisol exposure in scalp samples promoting inflammatory cytokine dysregulation

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Abstract

Background: The relationship between stressful periods and cutaneous manifestations are well known. The Pandemic crisis that occurred in recent years significantly increased skin stress reports worldwide.

Methods: We developed an accurate scalp and hair model evaluating the effect of cortisol application. Cortisol (10, 100 and 500 µM) or Sodium Dodecyl Sulphate (SDS) were resuspended in growth medium and applied to scalp for 24 hours (Group 1) or 48 hours (Group 2). Metabolic activity and interleukins secretion of IL-6, IL-8, IL-12 p70, IL-10, TNF and IL1-β were analyzed.

Results: SDS application severely reduced tissue viability as expected. Cortisol 10 and 100 µM did not induce a significative reduction compared to controls. However, Cortisol, 500 µM, reduced significantly viability of the samples. Cytokine screening revealed that although IL-6 and IL-8 were constitutively secreted in control samples and highly reduced by SDS application, a dose-response cortisol reduction was observed of both interleukins regardless of time application. IL-1β and TNF were detected in low amounts in all conditions tested. Secretion of IL-10 was inhibited by cortisol only after 48 hours while IL-12p70 was increased due to SDS and cortisol. These results showed the implication of TH1 and TH2 specific cells.

Conclusion: Cortisol has a significant role to play in scalp tissue stress and dysregulation which needs predictive and robust *in vitro* / *ex vivo* human tissue bioassays. We have, here, developed an innovative epidermis-dermis-hair follicle *ex vivo* model that mimics stress and body consequences on skin especially on inflammatory cytokine secretion.

Keywords: Cortisol; Stress; Neuroscience; Scalp; Pandemic;

1. Introduction.

Living with stress came to the forefront of our understanding of sensory science during Pandemic times. The Covid-19 crisis being a higher risk factor to generate stress for all populations and especially for older-age groups [1]. Numerous studies have proven effects of stress on skin but more research is needed and not enough models are available for proving cosmetic ingredients can help overcome skin stress [2, 3].

Cortisol is a steroid hormone synthesized from cholesterol and widely known as the body's stress hormone [4]. It has many functions in the human body and is not restricted to the primary nervous system, but also peripherally around the body including scalp and skin. Defence against infection is a major role, but dysregulation of the cortisol system, especially over a long chronic period can lead to deleterious effects and irritation in the dermal system. The role of hair follicle is considered important in the process of long term chronic scalp issues. Barrier function and keratinocyte turnover is already known to be perturbed during immunological events with production of cytokines stimulating the recruitment of immune cells [5, 6].

Both production and secretion of cortisol was regulated by the hypothalamus-pituitary-adrenal axis (HPA axis). Stress and emotions are processed by the brain amygdala which activates the HPA axis causing the release of cortisol.

Therefore, in the present study, we developed a hair follicle – epidermis – dermis model to mimic any negative effects of psychological stress and help to create a screening system which would be suitable for actives, products or treatments.

Our study highlights our *ex vivo* model as an accurate approach for testing cosmetic actives dedicated to scalp and hair care.

2. Materials and Methods.

2.1 Sample collection

All tissues used in this study were provided from human skin samples following ethical consent. Skin samples, which were surgical waste, were transported under optimal conditions to ensure and maintain tissue viability. CTIBIOTECH has a double accreditation from the French Ministry in charge of research for the preparation (AC-2018-3243 and DC-2018-3242) and conservation of elements derived from the human body with a view to their transfer for scientific purposes. The operation was carried out according to the strictest hygiene rules.

2.2 Ex-vivo model preparation

Explants were produced from scalp skin samples of female donors age range 61 to 71 years following planned elective surgery and donors were split randomly into 2 groups. All donors were collected during the Pandemic which was in itself a challenge. Only areas with high density of hair follicles (up to 100 hair follicles) were used. Punches of 8 mm² (Kai, Laboderm, Asnières-sur-Seine, France) were done on the full skin thickness and placed in 12-well insert plates (Corning, New-York, USA) according to CTIBiotech optimization protocols and media (**Figure 1**.).

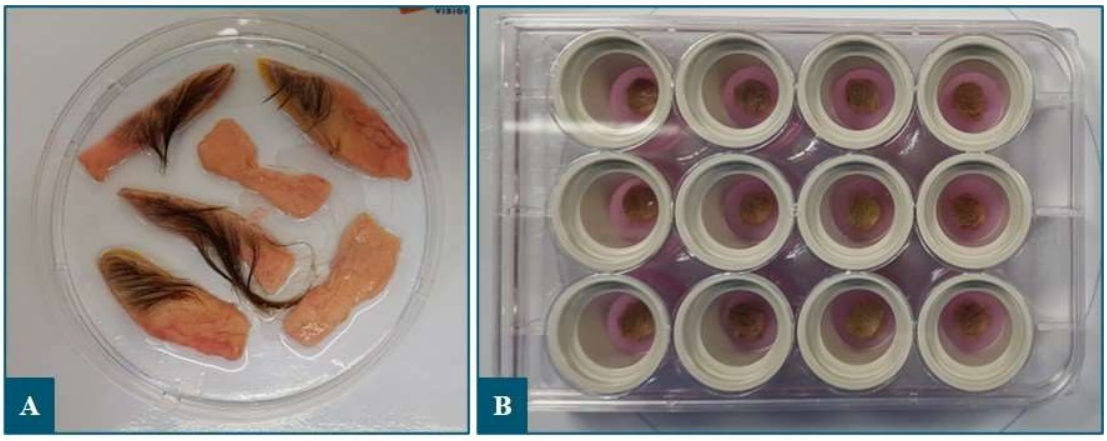

***Figure 1**., Ex-vivo scalp explants (B) produced from human skin sample (A).*
Areas with a high density of hair follicles were used.

Uniform sized *ex vivo* samples were made and placed into skin culture medium (CTIBIOTECH, Lyon, France) for maintenance before the testing phase. The scalp samples taken were classified as non-pathological with no presenting dermatological issues.
Samples were allowed to equilibrate for 24 hours prior to treatment.

2.3 Treatment application to samples

In order to assess effect, products were applied in systemic way resuspended in skin growth medium (CTIBIOTECH, Lyon, France) and prepared immediately before application. Skin explants were placed with the epidermis at the "air-lift" position. Group 1 was treated for 24 hours (**Figure 2**.) while group 2 was treated for 48 hours (**Figure 2.B**) to mimic a longer stress period. Treatment was not repeated during this time period. Different conditions were tested as follows: untreated condition corresponding to skin culture medium only (negative control), positive control using Sodium Dodecyl Sulfate at 3% during 30 min (SDS - Sigma-Aldrich, Saint-Quentin-Fallavier, France) and cortisol (Sigma-Aldrich) at 10 µM, 100 µM and 500 µM. The positive control was tested only on 24 hours application. For group 1, treatment was rinsed after 24 hours of application and explants maintained in skin culture medium until the end of incubation of group 2 (Day 3). Each condition was performed in triplicates.

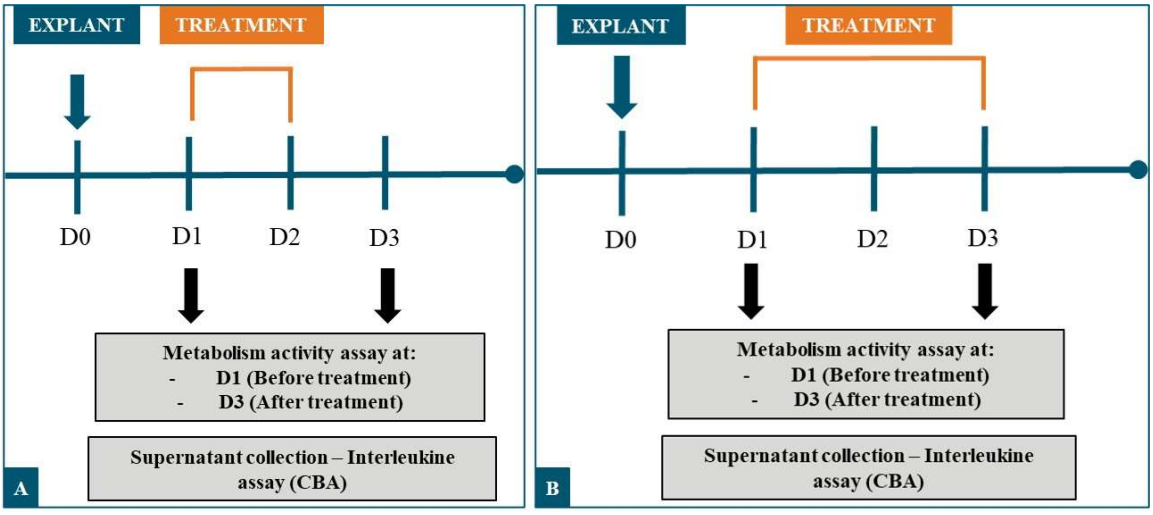

***Figure 2**., Timeline of experiment depending on the time of application of the treatment: 24 hours (A) or 48 hours (B).*

2.4 Metabolic activity analysis

Alamarblue™ Cell Viability reagent (Invitrogen, Carlsbad, Carlifornia) was directly added in each individual well and incubated for 4 hours. Supernatants were collected and transferred to a black-walled 96-well plate (Corning, New-York, USA). Fluorescence was quantified on a microplate reader (Tecan Spark®) at 550 nm, excitation and 590 nm, emission. This protocol was repeated before (Day 1) and after product applications (Day 3).

2.5 Cytokine levels

Following treatment steps, supernatants were collected and analyzed using Cytometric Bead Array (CBA) Human Inflammatory Cytokine kit from BD Biosciences on BD FACSVerse Cytometer (Becton Dickinson, Grenoble, France). The following interleukins were measured: IL-6, IL-8, IL-12 p70, IL-10, TNF and IL1-β.

3. Results.

3.1. Metabolic analysis of sample viability

Alamar blue analysis revealed that variation between samples before treatment was not wide, which is good for *ex vivo* tissue sampling and our processing techniques (**Figure 3A.**).
However, SDS application was extremely toxic to the *ex vivo* samples as expected (**Figure 3B**.). Although lower concentrations of cortisol did not cause any differences from the controls, the 500 µM concentration had a deleterious effect on the viability of samples (**Figure 3B**.).

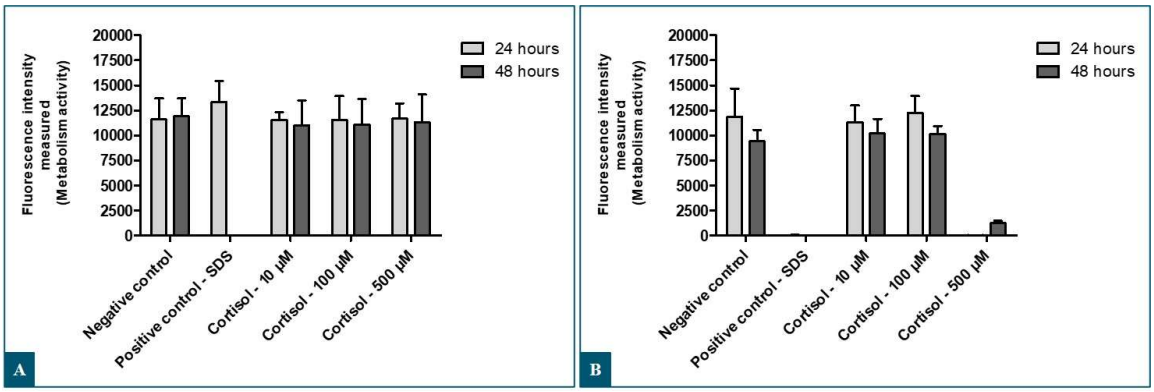

***Figure 3**., Alamar Blue quantification before (A) and after (B) treatment application (Day 1 or Day 3 – n=6)*

3.2. Investigation of key cytokines reveals the role of Cortisol

Cytokine analysis by CBA testing (Becton Dickinson system) investigated a range of cytokines known in the immunology of skin and scalp. This revealed interesting differences in expression of these regulatory molecules. Whilst some cytokines including IL-6 and IL-8 are known to have a constitutive (ongoing) expression throughout normal skin physiology, others are only active upon stimulation at the appropriate stage of immunology. In the current study, as expected IL-6 and IL-8 had important levels of expression in the control untreated samples. However, SDS, and all concentrations of cortisol disrupted this balance with reductions in these cytokines (**Figure 4**.). At 48 hours the response to cortisol was dose responsive.

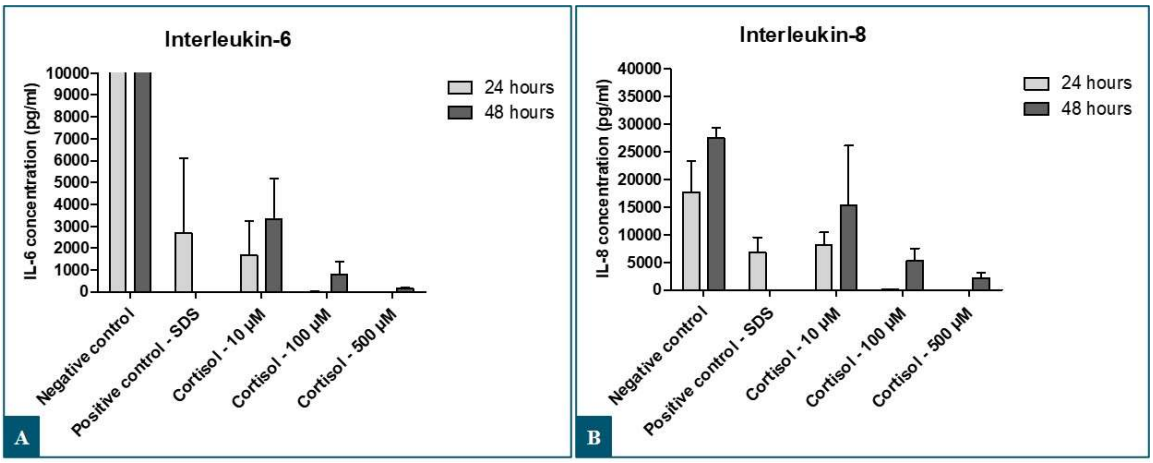

***Figure 4**. IL-6 (A) and IL-8 (B) Secreted cytokines from skin scalp explants after treatment applications.*

Whilst some cytokines require a longer time period to reach significant activity, it was interesting to observe that most other cytokines were not expressed in large quantities. In normal immunology with the cell-to-cell interactions this is a normal process not always requiring large quantities of the cytokine. For IL12-p70 SDS increased levels, but more interestingly cortisol dose-response increased the level of this cytokine (**Figure 5A**.). Interleukin-10 was more of a variety with no condition being statistically different from controls, with only a trend of reduction by SDS and cortisol at 24 hours and some recovery at 48 hours (**Figure 5B**.). Although TNF levels were very low also, cortisol did have some increases of this cytokine at higher concentrations, although this cytokine is known to require more time for secretion during immunological events (**Figure 5C**.). IL1-β was also not secreted much and although higher at 48 hours, was still a low level, even if a trend for reduction at 48 hours was seen with SDS and cortisol.

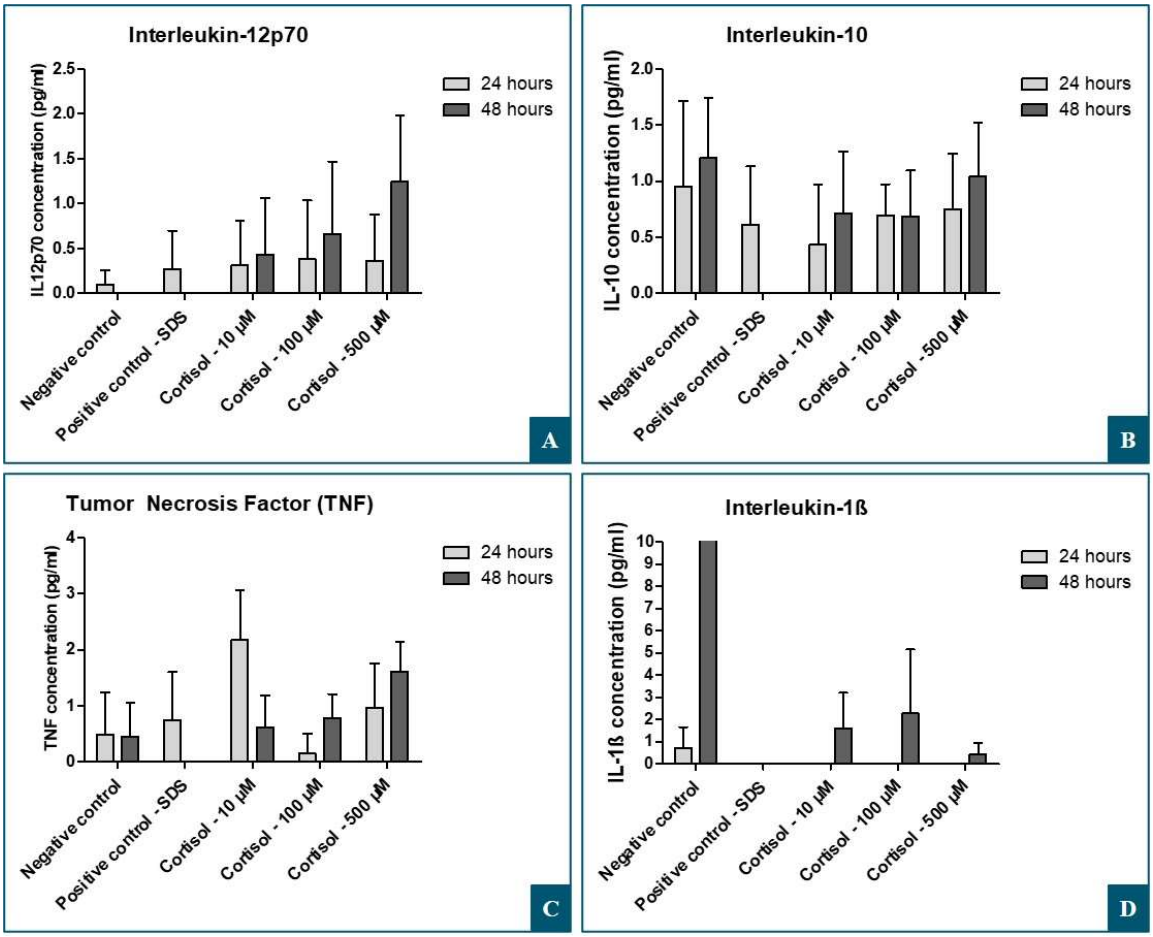

***Figure 5**. Dosage of IL-12p70 (A), IL-10 (B), TNF (C) and IL-1β (D) cytokines from skin scalp explants secretion after treatment applications.*

4. Discussion.

Studying the role of the brain on the other parts of the human body came to the forefront of science through research on fatigue and stress. However, anecdotal records of problems in other systems of stressed individuals from non-neurological clinics implicated brain related control systems as having a more system wide effect. For the skin it took some time for this to be translated, but it is now well known that scalp related issues can have a basis in stress [7]. The role of stress in skin conditions like atopic dermatitis and psoriasis are also known [3]. Further to that, during the high levels of stress seen in the COVID-19 pandemic, further evidence emerged [1]. Without doubt, cortisol, a potentially catabolic tissue damaging hormone, has a significant role to play in scalp tissue stress and dysregulation, with strong implications in hair, which warrants further investigation in the cosmetics and dermatology community [7, 8].

Here in this study we have shown that the role of cortisol is defined and has immunological interactional involvement. As with every organ, cytokines are involved in the normal cellular turnover. In the case of skin IL-6 and IL-8 are known to be constitutively expressed. Reduction due to SDS, a traumatic chemical was expected which is why it was chosen as a control. However, the dose-response reduction of metabolic activity by cortisol indicated a direct effect on cellular turnover kinetics. IL-12p70, IL-1β, IL-10, and TNF were produced in low amounts in all samples, with nearly all production of IL-1β being inhibited by SDS/cortisol (albeit low expression). IL-10 inhibition by cortisol required longer treatment of 48 hours which is expected since macrophage involvement is a process of 'commitment'. Resident naïve macrophages have been supplied to the skin environment as monocytes which then develop into tissue residing early macrophages. Such macrophages are then available for stimulation towards the TH1 or TH2 pathways. The sophisticated combination of cytokines stimulating is therefore key. Some involvement of IL-12p70 increases due to SDS and cortisol, would appear relevant as a strong TH1 stimulating event and reducing TH2. Therefore, the push towards TH1 during cortisol exposure seems implicated in increasing local inflammatory events. This is an important early stage in the recruitment of inflammatory cells to the site, since IL-12p70 is a potent regulator with a significant role in

cellular kinetics and later stimulation of other immune cells [9]. Around 25% of samples already had increased levels of IL-12p70 compared others, potentially indicating existing stress. Although the inflammatory response is linked to many factors, some internal and some external to the skin, the complex rise and fall of the different cytokines over a longer period can lead to a looping effect causing intermittent periods of scalp irritation. IL-6 and IL-8 are important in the allergy responses, since variations in IL-8 can trigger neutrophil recruitment and the implication here is important in corresponding with other studies related to stress [10, 11]. Interleukin-12p70 was expressed at low levels, though the increase over the longer treatment period was interesting since this cytokine has been implicated in the early stimulation phase of other cells to produce the much stronger acting cytokines, including interferon. With the current group of cytokines, there may have been a move to promotion of TH1 cytokine response. Long-term cortisol effects have also been reported in the elevation of IL-12 production [12].

Scalp dermatitis is a significant problem not to be underestimated. Like acne, it can cause severe embarrassment in sufferers, which leads to a vicious circle effect on the brain and therefore the skin. Cortisol as a small and distributable molecule is gaining ground in skin related research [13]. Understanding the location of the stress and effects could allow researchers to target the delivery of the cortisol and / or the activation of other related surface receptors to calm the effects and break the stress cycle. Some work in this area has moved forward in the pharmaceutical domain, but more is required on the cosmetics ingredients side [14].

5. Conclusion.

Testing and efficacy for advanced cosmetics remains on the major hurdles for proof of action and safety. Here we have created a new test for the scalp-hair-stress cycle, where cortisol is a major implicated factor. Scalp was severely perturbed by higher concentrations of cortisol and the speed (only 1 to 2 days) of action was an important factor indicating that even short periods of stress can affect the scalp quality.

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Conflict of Interest Statement.

NONE.

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