**The photoprotective properties of α-tocopherol phosphate against long-wave UVA1 (385 nm) radiation in keratinocytes *in vitro***

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# Supporting Information



**Fig.S1: The spectral outputs of the radiation sources.** The spectral output of the sources used in all the studies as measured with a Bentham spectroradiometer (details below in dosimetry section) from 280-460 nm.

**Table S1: Spectral waveband analyses of the radiation sources.** The spectral breakdown of each of the sources used in all the studies as measured.

|  |  |  |  |
| --- | --- | --- | --- |
| Source | Region | Wavelength (nm) | % of total irradiance |
| SSR | UVC | 250-280 | 0 |
| UVB | 280-320 | 12 |
| UVA | 320-400 | 88 |
| Visible | 400-500 | 0 |
| Total | 280-500 | 100 |
|  |
| 385 nm | UVC | 250-280 | 0 |
| UVB | 280-320 | 0 |
| UVA | 320-400 | 94.82 |
| Visible | 400-500 | 5.18 |
| Total | 280-500 | 100 |

**Table S2: The doses of SSR used and percentage reduction in absorbance for α-TP and α-T photostability study.** The equivalent doses (J/cm2) for each dose (SED) used to test the photostability of 0.5 mg/mL (1 mM) α-TP or 0.43 mg/mL (1 mM) of α-T. The percentage degradation compared to 0 SED as measured by UV spectrometry between 278-320 nm (n=3).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Dose (SED) | Exposure time  | Dose (J/cm2) | Abs. at 288 nm | % degradation of α-TP | Abs. at 291 nm | % degradation of α-T |
| 0 | 0 s | 0 | 0.92 ± 0.02 | 0 | 0.78 ± 0.08 | 0 |
| 10 | 70 s |  17.8 | 0.92 ± 0.02 | 0.03 | 0.81 ± 0.04 | 3.22 |
| 20 | 140 s |  35.6 | 0.93 ± 0.03 | 1.41 | 0.77 ± 0.82 | -1.61 |
| 30 | 210 s |  53.3  | 0.93 ± 0.02 | 1.92 | 0.73 ± 0.04 | -6.65 |
| 40 | 280 s |  71.1  | 1.01 ± 0.03 | 10.18 | 0.73 ± 0.11 | -6.95 |
| 50 | 350 s |  88.9  | 1.02 ± 0.05 | 11.34 | 0.67 ± 0.06 | -14.61 |



**Fig.S2: Tolerability of HaCaT keratinocytes when treated with (0.005-4,650 μM) of α-T diluted in 0.5% ethanol, 95% Dulbecco's modified eagle's cell culture medium (with FBS) at 37°C for 24 hours.** The α-T was well tolerated by the HaCaT keratinocytes when assessed using both the Alamar blue® (blue columns) and neutral red (red columns) cell viability assays. All the concentrations show viability ≥ 90%. Data represents mean ± standard deviation, (n=3).



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**Fig.S3: Pre-treatment studies on HaCaT keratinocytes. The generation of ROS in HaCaT keratinocytes at 2 min intervals after UVA1 irradiation over 1.8-2 h.** HaCaT were pretreated with 100 µM of α-TP and α-T for 24 h and subsequently re-incubated with 20 µM DCFDA for 45 min and then immediately washed with PBS and exposed to a UVA1 dose of 57 J/cm2. The TBHP was added into the reserved positive control wells at at concentration of 250 µM. Data represent mean ± SD (n=3).

**Table S3: The generation of ROS in HaCaT keratinocytes pre-treated with antioxidants**

|  |  |  |  |
| --- | --- | --- | --- |
| Condition  |

|  |  |
| --- | --- |
|

|  |
| --- |
| Mean (n=3) difference (a.u.) over 2 h compared to unirradiated control |

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| Positive control (TBHP) | 385 nm UVA1 (57 J/cm2) | 385 nm UVA1 (57 J/cm2) + pre-treatment (100 µM) |
| HaCaT + pre-αTP | -8,414,000 | -14,220,000 | -10,800,000 |
| HaCaT + pre-α-T | -7,685,000 | -13,020,000 | -9,908,000 |

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**Fig.S4: The DPPH radical scavenging/inhibition activity of α-TP (a), L-ascorbic acid (b), and α-T (c). All tested antioxidants show an ability to quench the DPPH radical as a measure of radical scavenging activity in a concentration-dependant manner.** All compounds demonstrated significant activity (p < 0.0001, linear regression analysis). The standard deviation was too small to be seen (n=3).