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Mini Review Article

Ciclosporin A Induces G2/M Arrest of the Cell Cycle in Cultured Human Keratinocytes

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Introduction

Although ciclosporin A (CsA) and tacrolimus are immunosuppressive drugs that inhibit T cell activation, it is possible that these drugs may work in part through direct action on the skin, independent of their action on T-cells. A number of lines of evidence indicate direct effects of CsA on epidermal keratinocytes. Several in vitro studies have reported a direct antiproliferative effect of CsA in cultures of proliferating epidermal keratinocytes 1,2. For example, CsA inhibits nuclear translocation of the transcription factor Nuclear Factor of Activated T cells (NFAT) via direct inhibitory effect on the phosphatase calcineurin in human keratinocytes3. In addition, CsA also inhibits TPA induced-cutaneous inflammation in severe combined immunodeficient mice that lack functional lymphocytes 4. Therefore, cell cycle progression of cultured human keratinocytes was investigated using flow cytometry.

Cells were cultured in T25 flasks and then treated with specified agents between 24 h and 120 h. Following treatment times, cells were trypsinised from flasks in 600-700µl of trypsin/EDTA for 10 min. This solution was then transferred to 1ml eppendorfs containing 70µl foetal calf serum (FCS). Cells were microcentrifuged, washed in phosphate buffered saline (PBS) and then repelleted. Finally, cells were resuspended as a single cell solution in 100µl PBS. 900µl of 70% ice-cold ethanol was added to each solution drop-wise whilst vortexing. Cells were kept in the freezer until ready for staining. The cell suspension was resuspended in 500µl PBS. To each sample 125µl of propidium iodide (PI): Triton X-100 solution (0.25mg/ml PI and 4.5% Triton X-100) and 50µl RNase (10mg/ml) were added and incubated for 30 min. Each sample was then analysed on a Fluorescence Activated Cell Scanner machine (FACScan) (Becton-Dickinson LTD; Oxford, UK). The DNA content of the cells was measured using a gated amplifier for FL2 (625nm) 5. For analysis, debris and doublets were excluded by using the FL2A/FL2W amplifier. Images were saved using Hewlett Packard (HP) Lysis II software. Pascal Hewlett Packard files were converted to DOS files by HP Reader (Becton-Dickinson; Oxford, UK), cell cycle analysis was performed on a PC using Multicycle software (University of Washington, Verity, USA).

Table 1 Ciclosporin A induces G2/M arrest of the cell cycle in cultured keratinocytes.

Experiment number	Treatment	% G0/G1	% S	% G2/M
1	DMSO (vehicle) 24 h	52.6	28.0	19.4
1	CsA (1 μM) 24 h	51.8	29.8	18.4
1	DMSO (vehicle) 48 h	62.9	12.1	24.9
1	CsA (1 μM) 48 h	58.4	17.2	24.4
1	DMSO (vehicle) 120 h	42.0	18.9	39.1
1	CsA (1 μM) 120 h	31.9	20.7	47.4
2	DMSO (vehicle) 24 h	52.6	27.2	20.2
2	CsA (1 μM) 24 h	55.0	23.4	21.6
2	DMSO (vehicle) 48 h	56.9	26.2	16.9
2	CsA (1 μM) 48 h	54.8	23.8	21.4
2	DMSO (vehicle) 72 h	53.7	28.3	18
2	CsA (1 μM) 72 h	41.3	25.0	33.8

Table 1 shows that CsA induced an increase in the proportion of cells in the G2/M phase of the cell cycle as compared to cells treated with a vehicle control at 120 h (Experiment 1) and at 72 h (Experiment 2). These results are obtained from 2 different experiments derived from 2 independent donors (Figure 1).

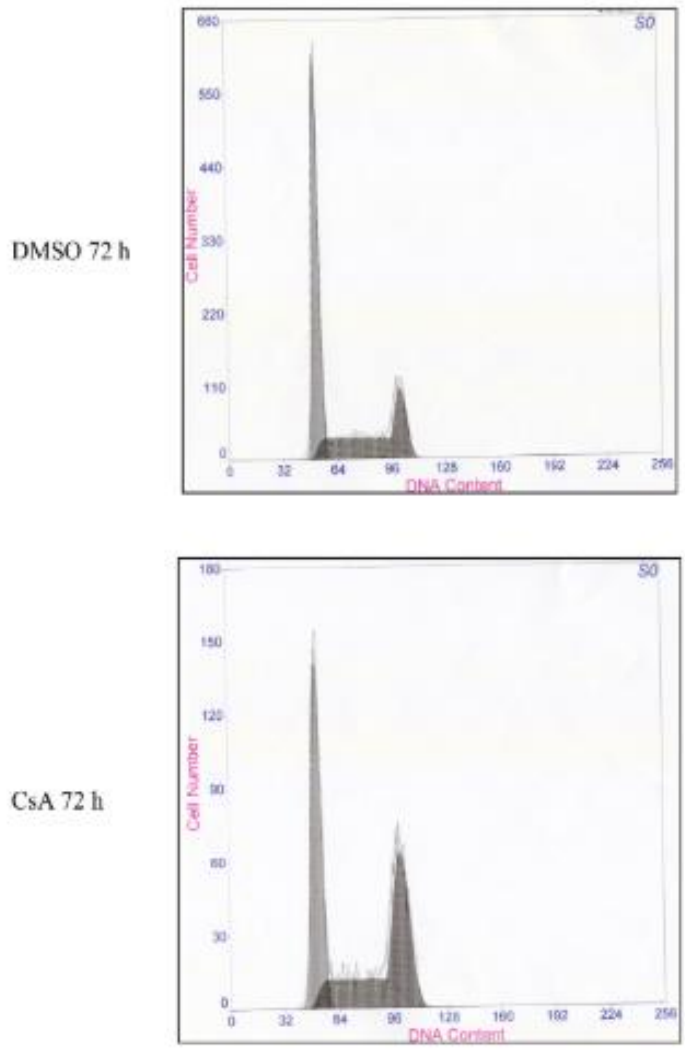

Figure 1: Ciclosporin A induces G2/M arrest of the cell cycle in cultured keratinocytes. Cell s were cultured in T25 flasks and then treated with CsA between 24 h and 72 h. Following treatment times, the cells were fixed and analysed on FACScan as described.

CsA at concentrations equivalent to therapeutic blood levels was reported to inhibit growth of cultured keratinocytes6. CsA was shown to arrest T cells in the G0/G1 phase of the cell cycle7. However, our flow cytometry results suggest arrest in the G2/M phase of the cell cycle, just prior to cell division following DNA duplication. This suggests that CsA may be able to affect the cell cycle at different phases or CsA effects might be cell-type specific. On the other hand, another study has showed that CsA acts via inhibitory effect on cultured keratinocyte cell cycle progression in the G1 phase of the cell cycle8. In conclusion, our findings might indicate that the effects of CsA on proliferation of cultured normal human keratinocytes are probably

related to direct effects on growth regulation of keratinocytes. Finally, these observations supports the possibility that the therapeutic effects of CsA in inflammatory skin disorders may be mediated, at least in part by direct effects of CsA in epidermal keratinocytes.

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