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The effect of Cyclosporine A on cardiomyocytes differentiation

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Abstract

Cyclosporine A (CsA) is a powerful immunosuppressive drug which significantly improved the success of organ transplantation; however, the major limiting factors for the drug's clinical use are its long and short term adverse effects. The present study was conducted to examine, in a dose-dependent manner, in a model of cardiogenesis, the effect of CsA on cardiomyocytes differentiation.

Keywords: Cyclosporine A • cardiomyocytes differentiation • HSP25 expression • cardiac actin expression

The immunosuppressive drug Cyclosporine A (CsA) is known to have pleiotropic effects at different levels (cell, tissue, organism). The immunosuppression is only one of the plethora of biological activities exhibited by CsA. Little is known about the biochemical events leading to well-known adverse effect on patients treated with CsA, the cytotoxicity and its mechanisms remains unclear.

In previous articles, we presented new effects of CsA at cellular level: GRP78 induction, MAP kinases ERK1 and ERK2 activation, HSF1 and HSF2 activation, enhancement of p53 level, hyperubiquitination of cellular proteins and HSP27 induction and hyperphosphorylation [1–3]. Davidson and Morange demonstrated that HSP25 and p38 MAP kinase pathway are involved in cardiomyocytes differentiation [4]. Duverger *et al.* also demonstrated the implication of HSP25 in the differentiation of PAM212 keratinocytes [5]. Other authors described the inhibitory effect of CsA on cell proliferation and differentiation and the inhibition of cardiac hypertrophy [6–9].

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In the present study we examined the effects of CsA in a model of cardiogenesis, the p19 cell line. p19 are multipotent embryonic carcinoma cell line; *in vitro,* in the presence of 1%DMSO and by day 6, rhythmically contracting cardiomyocytes arise in the interior of aggregates (colonies).

The aggregates were obtained using the 'hanging drops' method [10, 11] and plating on gelatine - coated coverslips.

On day 1, the cells were treated with: DMSO (1%), DMSO (1%) + CsA (1.25_M, 2.5 μM, 5 μM , 10 μM) and CsA μ5 μM, 10 μM).

On day 6, the percentage of the developed aggregates, the size of aggregates and the percentage of aggregates per dish containing a region which was beating and scored; cellular HSP25 and cardiac actin were stained with specific antibodies.

The analysis of results showed that (fig.1):

(1) at high concentrations (5 μM, 10 μM), CsA inhibits the cardiac actin expression and consequently the rate of beating aggregates; at 10 μM, the size of aggregates diminished
(2) HSP25 expression is not affected by 5 μM and 10 μM CsA or seems to be stimulated

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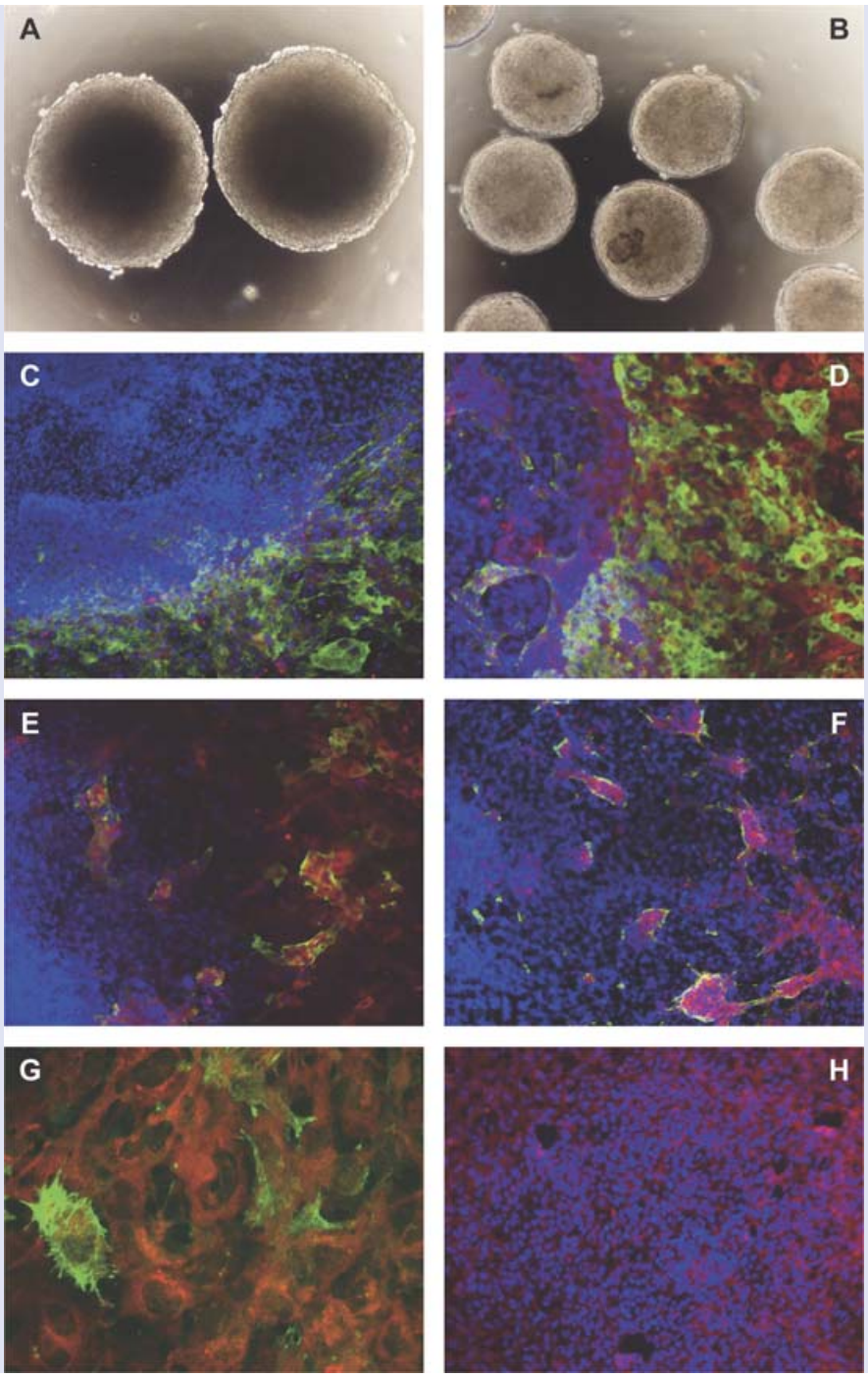

Fig. 1. A, B. Cell aggregates. **A.** Control (DMSO 1%). **B.** Cyclosporin A (CsA) 10μM. **C–H.** Patterns of HSP25 and myocardial actin during differentiation of cardiomyocytes (from carcinoembryonar C19 cell line). Red Cy3 (HSP25), green FITC (actin), blue Hoechst. **C.** Control, DMSO 1% (10x); **D.** DMSO 1%, CsA 1.25μM (10x); **E.** DMSO 1%, CsA 5μM (10x); **F.** DMSO 1%, CsA 10μM (10x); **G.** CsA 5μM (40x); **H.** CsA 10μM (10x)

and an intracellular aggregation of this protein might be observed;

(3) low concentrations of CsA (1.25 μM, 2.5 μM) do not significantly affect aggregates development and contractility.

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