Effect of the protein phosphatase inhibitors, okadaic acid and calyculin A, on dexamethasone-mediated inhibition of neutrophil apoptosis.

JOANNE M. COUSIN, CHRISTOPHER HASLETT and ADRIANO G. ROSSI.

Rayne Laboratory, Respiratory Medicine Unit, University of Edinburgh Medical School, Teviot Place, Edinburgh, EH8 9AG.

The onset of constitutive cell death by apoptosis in human neutrophils can be delayed by treatment with glucocorticosteroids such as dexamethasone [1-3]. The mechanisms underlying this steroid-mediated prolongation of neutrophil survival are however unknown. Okadaic acid and calyculin A, specific and potent inhibitors of protein phosphatases 1 and 2A, have been shown to influence the rate of apoptosis in different cell types [4, 5]. Moreover, protein dephosphorylation has been implicated as an essential step for glucocorticoid-mediated apoptosis in T-cell hybridomas [6]. In this study, okadaic acid and calyculin A were used to investigate whether phosphorylation/dephosphorylation plays a role in the intracellular signalling pathways controlling dexamethasone-mediated inhibition of neutrophil apoptosis.

Human neutrophils were isolated from the peripheral blood of healthy volunteers, as described previously [7]. Harvested neutrophils were resuspended in Iscove's DMEM supplemented with 5% autologous serum and 50 μg/ml penicillin and streptomycin, and finally cultured at 37 °C in a humidified 5% CO2-rich atmosphere. Neutrophils (3 x 10⁶) were incubated in the presence of various concentrations of okadaic acid (0.5 nM - 500 nM) and calyculin A (1 nM - 100 nM) both alone and in combination with dexamethasone (1 μM), in a final volume of 100 μl in flat-bottomed 96 well flexiplates. After 20 h in culture, neutrophils were harvested for assessment of morphology, viability (trypan blue exclusion) and recovery. Cytocentrifuge preparations of duplicate cell samples in each experiment were prepared, fixed in methanol, and stained with Diff-Quick. Slides were examined microscopically to determine the number of cells with apoptotic morphology in five fields of view (at least 500 cells were counted) on each preparation. Results were expressed as mean ± SEM for each preparation and analysed by analysis of variance followed by Student-Newman-Keuls post test. When p<0.05 (*), the results were considered to be significant.

Figure 1 demonstrates that okadaic acid (100 nM) had no significant effect upon the control rate of neutrophil apoptosis while reversing dexamethasone-mediated inhibition of neutrophil apoptosis at the concentration stated. We have previously shown that over a range of concentrations (0.5 nM-500 nM) okadaic acid has a bi-phasic effect; low concentrations inhibited whereas high concentrations promoted the rate of neutrophil apoptosis [6].

Figure 1. Effect of okadaic acid upon dexamethasone-mediated inhibition of neutrophil apoptosis.
Neutrophils were cultured for 20 h in the presence of dexamethasone (1 μM), okadaic acid (100 nM) or both. Data represent mean ± SEM of 4 experiments, each performed in duplicate.

Figure 2. Effect of calyculin A upon dexamethasone-mediated inhibition of neutrophil apoptosis.
Calyculin A (10 nM) also had no significant effect upon the control rate of neutrophil apoptosis while reversing dexamethasone-mediated inhibition of neutrophil apoptosis (figure 2). This observation was found to be consistent for the concentration range (1 nM - 100 nM) of calyculin A (data not shown).

Collectively, these results show that protein phosphatase inhibitors reverse dexamethasone-induced inhibition of neutrophil apoptosis. Furthermore, modulation of cellular phosphatases may be a fundamental mechanism underlying steroid-mediated prolongation of neutrophil survival and therefore may have important implications for the resolution of the inflammatory response.

We thank the University of Edinburgh and MRC for grant support.