THE INFLUENCE OF KYNURENINE, NEOPTERIN, AND NOREPINEPHRINE ON TUBULAR EPITHELIAL CELLS AND ALVEOULAR FIBROBLASTS

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ABSTRACT

We have examined the effect of kynurenine, neopterin and norepinephrine on epithelial cells and fibroblasts: the total DNA synthesis (by adding ³H-thymidine), the mitotic index, the amount of pathological mitosis and apoptosis. We have observed that kynurenine, neopterin and norepinephrine induce pathological mitosis and apoptosis of epithelial cells of kidney. The total DNA synthesis increases by the incubation of the epithelial cells with neopterin and norepinephrine. We have found an increase of pathological mitosis after addition of kynurenine, neopterin and norepinephrine to fibroblast cell culture. The total DNA synthesis is promoted by neopterin and norepinephrine, while kynurenine does not alter it. These data allow us to suggest that kynurenine, neopterin and norepinephrine promote epithelial cell damage leading to cell death through apoptosis, and can therefore be added to the factors, which promote the pathological mitosis of fibroblasts.

1. INTRODUCTION

Significant associations between exogenous and endogenous interferon—gamma induced neopterin formation and tryptophan degradation, with concomitant formation
of kynurenine, are observed in a variety of clinical situations in which cellular immune mechanisms are known to play a role, e.g., in viral infections (Wachter et al., 1979), acute cellular graft rejection (Margreiter et al., 1983), diseases associated with intracellularly living bacterial pathogens (Fuchs et al., 1984a), the acquired immunodeficiency syndrome (Fuchs et al., 1984b) and certain malignancies (Aulitzky et al., 1985). There exists also a close pathogenetic connection with kynurenine and neopterine, from one side, and nor­epinephrine, from other side (Rudzite et al., 1987a, 1989b). In clinical praxis it is important to evaluate the influence of such metabolites on cells in vivo. In our previous work we have demonstrated an increased serum concentration of kynurenine in patients with chronic mesangiproliferative glomerulonephritis (Rudzite et al., 1991).

We have now examined the effect of kynurenine, neopterin and norepinephrine on epithelial cells and fibroblasts.

2. MATERIAL AND METHODS

The epithelial cell culture of the kidney of dog (MDCK culture) and fibroblasts of human lung (HL culture) were used in this study.

After the cell adhesion (2 hours) we incubated the tissue culture with DMEM—
medium (Sigma) and foetal bovin serum (Gibco BRL) with addition of L-kynurenine (SERVA) (4, 10, 20μmol/L), D-erythro-neopterin (Schircks Laboratories) (5, 15, 30 nmol/L) or norepinephrine (SERVA) (2, 4, 8μmol/L) for 48 hours. We examined the total DNA synthesis by adding 3H-thymidine 2μCi/ml from the 47th till the 48th hour of our experiment. After 48 hours we isolated the cells using 0.02% trypsine and 0.02% EDTA, milipore filters (1,5 microns) and rinsed them with physiological solution. The filters were rinsed 3 times with 5% trichloraceticacid, fixed with absolute ethanol and dryed at 37°C. We recorded the 3H-thymidine incorporation in Packard chamber. We examined 5000 cells by light microscope and determined the mitotic index and the amount of pathological mitosis and apoptosis (Gang et al., 1967; Gstraunthaler, 1988). With every concentration of metabolite, we examined 3 samples. In control cultures, we examined all parametrs without addition of metabolites. The experimental studies have been made at the Microbiological Institute of Latvia, Riga.

Statistical analyses were done using Students t-test. P-values below 0.05 were considered to indicate significant differences. Data are expressed as mean ± SEM (standart error of the mean) (Weber, 1957).

3. RESULTS AND DISCUSSION

Table 1 shows the influence of kynurenine, neopterin and norepinephrine on fibroblasts in HL cell culture. We have observed an increase of pathological mitoses after addition of kynurenine (4, 10 and 20μmol/L), neopterin (15, 30nmol/L) and norepinephrine (2, 4 and 8μmol/L) to fibroblast cell culture. Only norepinephrine (2, 4, 8μmol/L) induces the apoptosis of fibroblasts. The incorporation of 3H-thymidine in fibroblasts (total DNA synthesis) was promoted by neopterin (5 and 15 nmol/L) and norepinephrine (2 and 4 μmol/L), while kynurenine had no effect.

In our previous investigations we found an increased 3H-thymidine incorporation in fibroblasts after addition of L-kynurenine to M-22 cell culture (Rudzite et al., 1992). It it has also been reported, that the mesangial cells can be tranformed to myofibroblasts.