Polyfurancarbonic Acid Inhibits Proliferation and Induces Apoptosis of Cultured Human T Lymphocytes *in Vitro*

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 127, No. 6, pp. 672-674, June, 1999
Original article submitted December 9, 1998

Polyfurancarbonic acid dose-dependently suppresses proliferation and induces apoptosis of human peripheral blood T lymphocytes *in vitro*. This finding indicates its possible involvement in the pathogenesis of lymphopenia during chronic renal failure.

Key Words: *apoptosis; proliferation; lymphocytes; polyfurancarbonic acid*

In 1986, chromatography of serum samples from uremic patients revealed the presence of a compound associated with plasma proteins. The mass spectrum of this compound corresponded to 3-carboxy-4-methyl-5-propyl-2-furan propionic acid with a molecular weight of 268 Da [3]. Metabolites of polyfurancarbonic acid (PFCA) are present in vegetables and fishes and can be synthesized in the body [2].

The concentration of PFCA in the serum of patients with chronic renal failure (CRF) correlates with the stage of this disease [1,4].

Albumin-associated metabolites (*e.g.*, PFCA) cannot be efficiently removed by hemodialysis and are accumulated in the serum of uremic patients [7]. There are data showing the involvement of PFCA in death of erythroid progenitor cells (by the mechanism of apoptosis) and in the inhibition of mitochondrial respiration in patients with CRF [6,8]. However, the effects of PFCA on the functional activity of human lymphocytes received little attention. Here we studied the effects of PFCA on proliferation and apoptosis of lymphocytes.

MATERIALS AND METHODS

Peripheral blood mononuclear cells of healthy donors isolated on a Ficoll-Verografin density gradient (1.077 g/ml) were cultured in RPMI-1640 medium (N.F. Gamaleya Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences) containing 10% fetal bovine serum (Sigma), 2 mM glutamine, and 10 mM HEPES (Sigma). Phytohemagglutinin (PHA, Vasto) in a concentration of 10 μg/ml was used as the mitogen. The cells (3×10^6 per ml) were incubated in 96-well plates (Sigma) at 37°C and 5% CO_2 for 72 h. To some wells, PFCA (kindly provided by Prof. G. Spiteller, Bayreuth University) in doses corresponding to its serum concentration was added. ^{3}H-Thymidine (specific activity 1 mCi/ml, Izotop) was added to the culture after a 68-h incubation to study the proliferative activity. The cells were precipitated on filters with a harvester. Radioactivity was determined by the number of scintillations induced by the tritium β-decay. The results were analyzed by the index of stimulation (IS):

$$IS = \frac{\text{cpm of stimulated cells}}{\text{cpm in control}}$$

Apoptosis was examined by flow cytometry using a modified method with propidium iodide [5].

To this end, peripheral blood lymphocytes isolated on the density gradient were incubated in a complete medium containing 10% fetal bovine serum and 10 μg/ml PHA in 24-well plates (3×10^6 cells/ml). Dexamethasone (10^{-3} mg/ml) was added to some wells,

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0007-4888/99/0006-0613$22.00

TABLE 1. Effects of PFCA on Proliferation and Apoptosis of T Lymphocytes ($M\pm m$, n=15)

Parameter	Control	PHA, 10 μg/ml	PHA, 10 μg/ml+ dexametha-sone, 10^{-3} mg/ml	PHA, 10 μg/ml+PFCA, μg/ml			
				20	50	100	150
IS		73.0±4.2	65.0±2.3	54.0±2.9	34.8±5.7	19.7±4.6*	10.9±2.6*
Relative content of apoptotic lymphocytes, %	3.0±0.1	8.0±0.3	50.0±1.5	55.0±1.6	59.0±1.2	63.0±0.8*	65.0±0.5*

Note: *$p \leq 0.05$ compared with the control.

and PFCA in doses corresponding to serum concentrations in patients with CRF was added to others. After 72-h incubation at 37°C and 5% CO_2 the cells were treated with 0.1% hypotonic sodium citrate and Triton X-100, stained with propidium iodide, and analyzed in a Becton Dickinson flow cytometer by the percent of stained cells in the hypodiploid peak (DNA content below standard diploid level) on a histogram of the 3rd fluorescence (Fig. 1). The results were analyzed by Student's t test.

RESULTS

In 15 healthy donors, the maximum values of IS and the lowest incidence of apoptosis were observed in culture with PHA (Table 1). Dexamethasone decreased IS and increased the incidence of apoptosis. Incubation with PFCA in doses of 20-150 μg/ml corresponding to serum concentrations in patients with CRF of various stages dose-dependently suppressed proliferation of lymphocytes and increased the percent of apoptotic cells [7].

Thus, we demonstrated the interrelation between serum concentration of PFCA in uremic patients and its effects on lymphocyte proliferation and apoptosis. These findings confirm the involvement of PFCA in the induction of lymphopenia and the development of secondary immunodeficiency in patients with CRF.

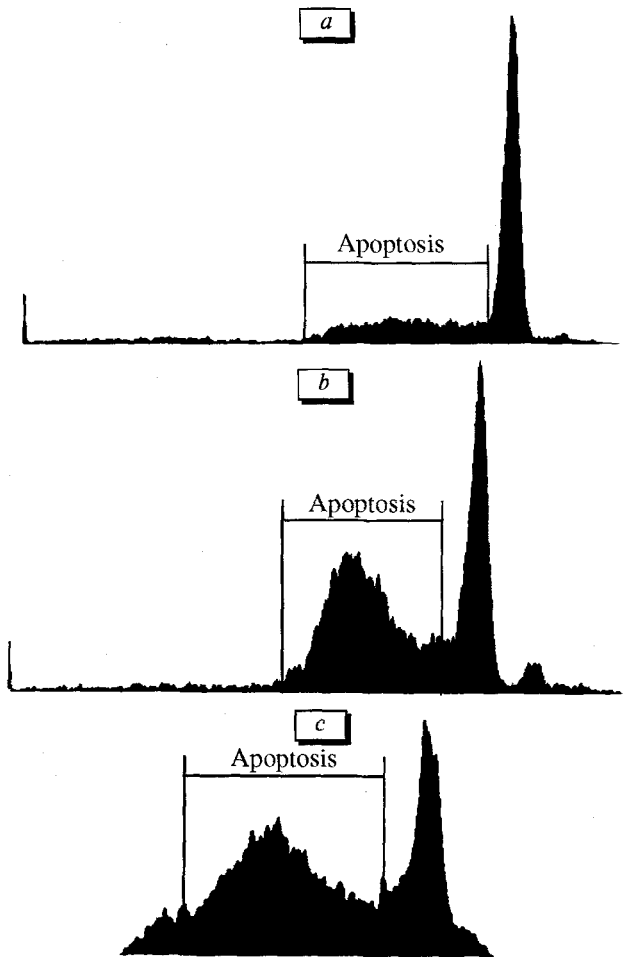

Fig. 1. Quantitative flow cytometry of apoptotic lymphocyte in *in vitro* culture stained with propidium iodide: *a*) lymphocytes cultured with phytohemagglutinin (PHA); *b*) lymphocytes cultured with PHA and dexamethasone; and *c*) lymphocytes cultured with PHA and polyfurancarbonic acid.

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