

Cytotoxic Effects of Colicins E1 and E3 on *v-myb*-Transformed Chicken Monoblasts

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Abstract. Colicins show a considerable cytostatic activity, which is much less known and understood than their killing activity targeting bacteria of the *Enterobacteriaceae* family. In this communication, the cytotoxic effects of colicins E1 and E3 on *v-myb*-transformed chicken monoblasts BM2 are presented. We detected clear reduction of the viable cell number induced by colicins E1 and E3, occurring without apparent changes in cell cycle profiles. The level of inhibition was proportional to the colicin concentration within the limits of 0.5–1.25 µg/ml. This result documents that colicins produced by *Enterobacteriaceae* exert their cytotoxic effects on leukemic cells.

Colicins are proteins produced by many strains of *Escherichia coli* and related species of the family *Enterobacteriaceae*; e.g. 41% of human *E. coli* strains are colicinogenic (Šmarda and Obdržálek, 2001). Discovered by Gratia in 1925, they have been thoroughly studied for dozens of years (for recent reviews see Braun et al., 1994; Šmarda and Šmajš, 1998). It is likely that only a part of colicin types has been found so far. In addition, colicins of new types can also be “tailored” from their functional compartments by techniques of recombinant DNA.

It is much less known that certain natural bacteriocins, mainly colicins, can inhibit tumor cells. The first experimental evidence of this phenomenon was gained as soon as 23 years ago, when a profound cytotoxic effects of colicin E3 on human HeLa cells was reported (Šmarda and Obdržálek, 1977). Later, blood tumor cells were also exposed to a colicin giving equal results (Farkas-Himsley and Kuzniak, 1978). This report demonstrated a cytotoxic effect of a poorly characterized colicin product of the *E. coli* strain HSC10 on murine ascitic lymphatic leukemia cells EL-4. The research group of Farkas-Himsley performed a variety

of further experiments using this colicin, until 1981. Unfortunately, all of their promising results were obtained with only partially purified colicin preparations. Using thorough protein purification it was shown that the anti-neoplastic activity was due to verotoxin 1, tightly attached and hardly separable from the HSC10 colicin preparations (Farkas-Himsley et al., 1995).

Colicin E3 at the concentration 1 mg/ml and higher was shown to inhibit proliferation of murine leukemia cells P388 in a dose- and time-dependent manner (Fuska et al., 1979). It obviously had a toxic effect on these cells. Interestingly, cell proliferation was stimulated at lower colicin concentrations. In addition, colicin E3 decreased the viability of three lines of murine lymphoma and plasmocytoma cells by 23%–51% in 100 min. Colicin D appeared to be equally efficient in these cell cultures. Similarly, a direct proportionality between the colicin D effect and its dose was observed. An even more dramatic effect was induced by colicin E2 (decrease of viable cell number by 40%–55%) and colicin A (decrease by 53%–58%) (Šmarda and Oravec, 1989). Colicin A treatment of mice with transplanted LP-2 plasmocytoma significantly prolonged their survival (Oravec and Šmarda, in preparation).

In the light of these pilot experiments, we tested a possible effect of two pure colicins on proliferation of tumor cells of another model system: the *v-myb* oncogene-transformed avian monoblasts BM2.

Material and Methods

Cells

BM2 cells are chicken monoblasts transformed with the *v-myb* oncogene of avian myeloblastosis virus (Moscovici et al., 1982). The cells were cultivated in humidified 10% CO₂ atmosphere as described (Zemanová and Šmarda, 1998).

Colicins

Industrially produced colicin E1 (M_r: 57.300) and colicin E3 (M_r: 58.000) were applied as pure substances. Colicin E1 was a commercial product of Sigma Chemicals Co., St. Louis, MO; colicin E3 was produced at the Institute of Sera and Vaccines, Prague, Czech Republic. For preparation of stock solutions the

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colicins were diluted in deionized water at a concentration 30 µg/ml.

Proliferation test

2×10^6 exponentially growing BM2 cells were seeded in 5 ml of Dulbecco's Modified Eagle's Medium (Sigma Aldrich Co. Ltd., Irvine, UK) containing fetal calf serum (5%) and chicken serum (5%) (Life Technologies Ltd., Auckland, New Zealand). At time zero, colicins E1 or E3 (0.5 and 1.25 µg/ml) were added; no substance was added to controls. To control the specificity of colicin effects, cultures treated with equal weight concentrations of bovine serum albumin (BSA) (M_r : 69.000) were also included. All cultures were incubated at 37°C for 96 h. At each of 24-, 48-, 72- and 96-h incubation intervals the number of viable cells was counted in four parallels using a hemocytometer. Four sets of experiments were run with each colicin. The activity of colicins in the culture medium was checked after 96-h incubation; they remained active throughout the experiment.

Cell-cycle analysis

2.5×10^6 BM2 cells were treated with either colicin E1 (1 µg/ml) or colicin E3 (1 µg/ml), or left untreated for 3 days. The cells were then pelleted, washed with phosphate-buffered saline (PBS), fixed in 70% ethanol, and stored for 24 h at 4°C. The fixed cells were stained with propidium iodide and processed for flow cytometry as described (Šmarda et al., 1999). DNA content was measured using the FACS Calibur system (Becton Dickinson, San Jose, CA). 2×10^4 cells were analyzed in each sample and the percentage of cells in each phase of the cell cycle was determined using the MoFit LT 2.0 software. Two independent experiments were performed.

Results

The effect of colicin E1 on BM2 cells was tested in concentrations 0.5 µg/ml and 1.25 µg/ml (Fig. 1A). In both concentrations colicin E1 clearly decreased the number of viable BM2 cells in the culture. The maximum effect was reached when the cells were exposed to colicin E1 (1.25 µg/ml) for 48 h. The number of living cells was decreased by more than 61% under these conditions. During prolonged colicin E1 treatment the inhibition rate was slightly lower, reaching about 35%. In each interval, the level of colicin E1 inhibition was dose dependent.

The suppressive effect of colicin E3 on the growth of BM2 cells was even more pronounced (Fig. 1B). The decrease of viable cells by colicin E3 was also dose dependent, similarly to the effect of colicin E1.

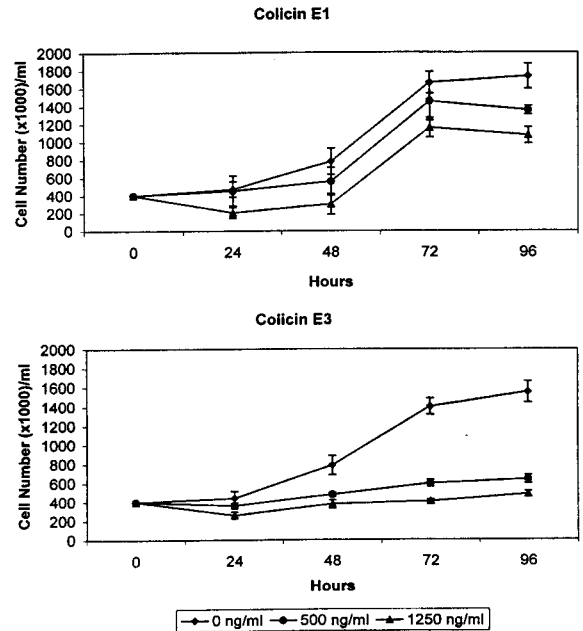


Fig. 1. Growth curves of *v-myb*-transformed monoblasts. BM2 cells were treated with colicin E1 (A) or colicin E3 (B) in indicated concentrations for four days. The number of viable cells was measured daily using a hemocytometer. Data representing the mean and standard error for four independent experiments are shown.

However, the maximum decrease of living cells (by 70%) induced by colicin E3 was reached at 1.25 µg/ml concentration following 72- and 96-h treatment. The specificity of colicin effects on BM2 cells was controlled by bovine serum albumin, a protein of only slightly higher molecular weight than colicins. The cells were exposed to this protein at the same concentrations as those of colicins resulting in absolutely no effect on cellular viability (not shown).

Next, we wished to investigate whether colicins exert any specific effects on cell-cycle profiles of BM2 cells. Therefore, we treated BM2 cells with colicin E1 and E3 under those conditions giving clear inhibitory effects. Then, the nuclei were stained with propidium iodide and DNA content was measured by flow cytometry. We detected a slight decrease in G1-phase cell number caused by both colicin E1 and E3 and an increase in S-phase cell number (Table 1). However, the cell-cycle changes of BM2 cells induced by colicins seem to be too weak to explain the clear decrease in the number of viable cells shown in Fig. 1. It is likely that colicins induce death processes in a certain fraction of BM2 cells and the dying cells exit the cell cycle in any phase. We did not observe any apoptotic DNA fragmentation in BM2 cells treated

Table 1. The cell cycle of *v-myb*-transformed monoblasts is not significantly affected by colicins.

Untreated			Colicin E1			Colicin E3		
G1	S	G2/M	G1	S	G2/M	G1	S	G2/M
72%	14%	14%	66%	19%	15%	67%	17%	16%