Effect of colicin E3 on leukemia cells P 388 in vitro

J. Fuska, Alžbeta Fusková, J. Šmarda[1] and J. Mach[2]

Department of Environmental Chemistry and Technology, Slovak Technical University, CS-880 37 Bratislava (Czechoslovakia), 3 July 1978

Summary. Proliferation of murine leukemia cells P388 is stimulated by less than 1.0 mg/ml colicin E3, while being inhibited by higher concentrations. By 1.6 mg/ml colicin E3, uptake of thymidine into cold TCA-precipitable fraction is decreased by 59% during 24 h, uptake of uridine by 29%.

Colicin E3, described as a specific bacterial endo-ribonuclease[3], exerts – even in low concentrations – a keen cytotoxic effect on normal mammalian cells in tissue cultures; its action on cells of the lines HeLa[4] and L[5] has been described. LD_{50} for HeLa-cells is about 30 times lower than LD_{50} for cells L. Analogous effect of colicin E3 on human cancer cells MDA 231 MB in vitro was also noted[6].

As the next step in these investigations, we examined the effects of treatment of mouse leukemia cells P388 in suspensions by colicin E3. The purpose was not only to extend the previous observations on a further tumor line to get comparative data, but also – and mainly – to follow the interference of colicin E3 with 2 elementary biochemical functions of these eucaryotic cells; with their DNA- and RNA-synthesis.

Leukemia cells of the line P388 were gained from tumors of mice (strain DBA/2) on the 7th day following implantation; media and procedures of cultivation were published[7].

A standard, highly purified substance of colicin E3 (CA 38) was used throughout the experiments. Its purity was tested as stated elsewhere[5]. Lyophilized preparation in evacuated ampoules was kept at +4 °C until used. Immediately before use, water solution of 17.5 mg/ml was prepared; specific activity of the solution was 1×10^{12} lethal units (l.u.)/ml (using bacterial cells *Escherichia coli* C6 as indicator), corresponding to specific activity of 5.71×10^7 l.u. per 1µg of the substance. To individual cell suspensions in test-tubes, colicin E3 solution in final concentrations of 0.4 mg/ml, 0.8 mg/ml, 1.6 mg/ml and 3.2 mg/ml was added, rendering the multiplicities of 5.48×10^4 l.u./l cell, 1.096×10^5 l.u./l cell, 2.19×10^5 l.u./l cell and 4.38×10^5 l.u./l cell, respectively. To control suspensions, an equal volume of sterile distilled water was added.

After 0-, 24-, 48- and 72-h cultivations at 37 °C, 10 µl volumes were removed from each suspension and cell counts in Bürker's microscopic counting chambre were made. In the same samples, the portion of dead cells was estimated after erythrosine staining[7]. In parallel experiments, the uptake and incorporation of thymidine-2-^{14}C into DNA and of uridine-^{14}C(U) into RNA of proliferating cells was followed[8,9].

During 72 h after seeding, the murine leukemia P388 cells multiplied in an almost linear proportion to time; within this interval, their proliferation was distinctly inhibited by 3.2 mg/ml and 1.6 mg/ml colicin E3 in a direct relation to the interval of treatment (figure 1). Even at the highest concentration, no cell lysis occurred (as opposed to distinct lysis of cells HeLa and L by colicin E3 multiplicities 1–2 orders lower).

After 24 h, uptake of thymidine was decreased by 37% by 0.8 mg/ml colicin E3; 2 mg/ml and higher concentrations caused also a remarkable decrease of uridine uptake, 3.2 mg/ml concentration by 59% (figure 2).

Thymidine uptake being distinctly inhibited already by 2.5 times lower colicin E3 concentration than uptake of uridine, it seems that DNA-synthesis in P388 cells is depressed prior to RNA-synthesis. However, with respect to the interference of colicin E3 with the ability of both epithelial (HeLa) and connective tissue (L-fibroblasts) cells to attach to glass or to stay attached[5], it is reasonable that in both these cell lines, as well as in leukemia P388 cells, the primary effect of this colicin may be directed to the cell surface, i.e. to plasma membrane, causing some specific change in it. In cells HeLa and L, this change is followed by detachment and autolysis, in P388 cells it is reflected – among other ways – in decreased rate of DNA- and RNA-synthesis and thus in inhibition of proliferation, but – as was shown – cells in this condition may still retain viability. Stimulation of proliferation at low concentrations could also be due to primary plasma membrane excitation. Thus our results are preliminarily not in favour of the identity of colicin E3 biochemical target in pro- and eucaryotic cells. At least in P388 cells, colicin E3 probably does not provoke its effect typical of sensitive cells of bacteria, i.e. specific

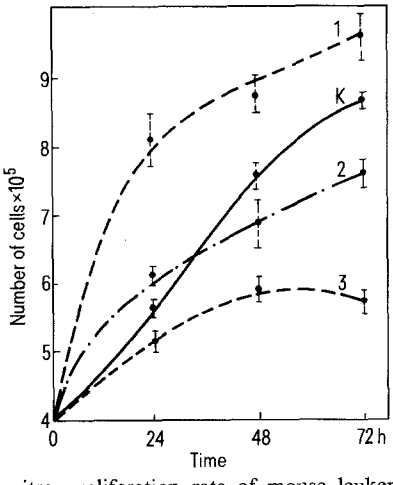

Fig. 1. In vitro proliferation rate of mouse leukemia cells P388 treated with various concentrations of colicin E3. K: control, 1: 0.8 mg/ml, 2: 1.6 mg/ml, 3: 3.2 mg/ml.

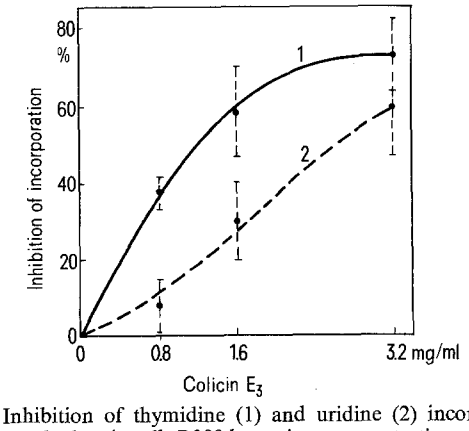

Fig. 2. Inhibition of thymidine (1) and uridine (2) incorporation into mouse leukemia cells P388 by various concentrations of colicin E3 during 24 h.

cleavage of a 3'-OH terminal fragment of 49 nucleotides from rRNA-molecules[10], in spite of the presence of both 18S- and 16S-rRNA. It also probably does not directly hit cellular DNA-molecules. Its effect may be characterized as cytotoxic rather than cytostatic.

In sensitive bacteria, colicin E3 - similarly as other ones - developes its effect in 2 phases: the first, bacteriostatic one (started immediately through adsorption on plasma membrane receptor), is succeeded in about 30 min by the second, bactericidal one[11]. It is well possible that in leukemia cells P388, the effect of E3 is limited to an analogy of the first one.

In this context, the substantially lower killing of P388 cells than of HeLa- and L-cells by colicin E3 is worthy of notice: while about 1×10^4 l.u. per cell are sufficient to kill 100% of cells HeLa, about 3×10^5 l.u. per cell for killing 100% of cells L during 3 days[5] and about 7.4×10^5 l.u. per cell for killing 29% cells of a human mammary carcinoma MDA 231 MB during 1 day[6], about 4.4×10^5 l.u. per cell suffice to kill only 1.7% of leukemia P388 cells in 3 days, although under different experimental conditions. This difference of 2 ten-orders is related to similar sensitivity differences found for most known cytostatics.

Preliminarily, pure colicin E3 appears to be nontoxic for laboratory animals and its pyrogenicity is very low. Thus it seems plausible to analyse its effect on tumor cells, especially on cells of other tumor types, in greater detail.

1 Department of Biology, Medical Faculty, J.E. Purkyně University, Brno.
2 Institute of Sera and Vaccines, Praha.
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A histochemical study of denervated marsupial hemidiaphragm

M.A. Khan[1]

Department of Anatomy, University of Queensland, St. Lucia (Queensland, 4067 Australia), 10 July 1978

Summary. The marsupial (quokka) hemidiaphragm showed postdenervation hypertrophy and subsequent atrophy. The type II muscle fibres hypertrophied up to 20 days postoperation and then regressed. However, the type I fibres hypertrophied throughout the experimental period (100 days) studied. Unlike denervated eutherian hemidiaphragm, fibre-splitting was absent in the denervated marsupial muscle. An enhancement of the ATPase reaction in the denervated type I fibres may be due to dedifferentiation. Presumably innervation exerts a 'negative control' and prevents increase of type I fibre size in the normal hemidiaphragm.

Vertebrate skeletal muscles atrophy when deprived of their innervation[2,3]; however, denervated rat hemidiaphragm shows a transient phase of hypertrophy for up to 10 days followed by atrophy[4-14]. Biochemical[6,7] and ultrastructural[8] studies confirmed an increase in contractile and sarcoplasmic proteins during hypertrophy. Further, the increase and decrease of proteins during hypertrophy and atrophy respectively paralleled the time course of an increase and decrease of RNA in the eutherian hemidiaphragm[7]. The purpose of this investigation was to evaluate the response of marsupial hemidiaphragm after denervation and to determine the histochemical behaviour of the 2 major muscle fibre-types sequentially.

Materials and methods. 12 quokkas *(Setonyx brachyurus)* of both sexes weighing between 1.97 and 3.45 kg were used in this study. The animals were anaesthetized with ether and intrathoracic denervation was performed and a 2 mm segment of the left phrenic nerve was removed[11]. On 4, 10, 20, 40, 60 and 100 days after phrenictomy, the normal innervated and the denervated muscles were removed carefully, cleared of all extraneous tissue and weighted. Small muscle blocks were excised from the normal and denervated hemidiaphragm muscles, and covered with talcum powder[15]. 10 µm frozen sections were cut on a cryostat and then incubated for the myofibrillar ATPase reaction[16].

Results. Compared with the innervated contralateral controls, the wet weight of denervated quokka hemidiaphragm increased: by 33.3% on day 4, 35.0% on day 10, 14% on day 20, 50% on day 40 and 12% on day 60. Thereafter its atrophy was 53% on the 100th day. Figure 1 shows that sizes of type I (pale staining) and type II (darkly stained) muscle fibres are similar. Unlike that seen in the rat hemidiaphragm[13,18], here the type I fibres were present in groups. Both type I and type II muscle fibres hypertrophied up to 20 days postdenervation (figures 2 and 3). Thereafter type II fibres regressed and atrophied; however, some hypertrophied and were still present on the 100th day (figure 4). The great majority of type I fibres hypertrophied throughout the experimental period (figures 2-4). Fibre splitting was absent in the denervated marsupial muscle during the postdenervation period studied (figure 4). In this denervated muscle, number of type IB (those with moderate ATPase reaction) appeared increased (figure 3). On the 100th day after surgery, all type I fibres displayed higher ATPase reaction (figure 4).

Discussion. Postdenervation hypertrophy of varying degree was evident in the denervated quokka hemidiaphragm up to 40 days. A previous report[11] also showed a 25-30% hypertrophy around day 40 in the denervated quokka hemidiaphragm; however, they did not study this denervated muscle prior to day 10. Here 33% and 35% hypertrophy of the hemidiaphragm were recorded on the 4th and 10th days, respectively, after phrenectomy.

As shown previously in the denervated rat hemidiaphragm[13], the type II fibres of quokka hemidiaphragm also hypertrophied up to 20 days postdenervation and then regressed. However, some hypertrophied type II fibres of this marsupial muscle were present in the atrophied tissue