Comparative Multiparametric Analysis of HeLa and RD Cell Culture Reactions to Solcoseryl

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Reactions of continuous HeLa and RD cell cultures and their nuclear and nucleolar apparatus to addition of solcoseryl into the medium were studied. The monolayer density, proliferation activity, percentage of dead cells, RNA and DNA content in the nuclei and nucleoli, number of nucleoli in the nuclei, cell distribution in the population by the number of nucleoli in the nuclei, volume and complete surface area of the nuclei and nucleoli, and the nucleolar/nuclear ratio were evaluated. The cultures differently reacted to solcoseryl in the medium at the population and cellular levels of their organization. By the results of multiparametric analysis of the reactions of cells and their nuclear and nucleolar apparatus, solcoseryl can be referred to bioactive substances with characteristics of a factor regulating cell population growth.

Key Words: *cell culture; population; nucleus; nucleolus; solcoseryl*

Continuous cell cultures are convenient models for evaluation of the effects of active compounds on cells and cell populations. We studied the reaction of Hep-2 cell culture to addition of Na^+- and Ca^{2+}-double-stranded RNA to the medium [3] and compared the reactions of cultured cells [2]. The presence of these bioactive substances in culture medium suppressed proliferation and other functions of cells; Ca^{2+}-double-stranded RNA exhibited higher activity. The results of this study prompted evaluation of cultured cell reactions to solcoseryl. Solcoseryl is a drug of biogenic origin, deproteinated hydrolysate of blood from sucking calves [6]. Solcoseryl attracted our attention because of its characteristics, reported by the manufacturer company (ISN). Among other things, it stimulates cell proliferation, reparative and regenerative processes, collagen synthesis, oxidative phosphorylation, oxygen consumption, and glucose transport to metabolically exhausted cells. Solcoseryl is widely used in medical practice, primarily for stimulation of regeneration processes in post-burn therapy of the skin [4,6,7]. We found no reports about the effects of solcoseryl on cultured cells and cell populations.

We carried out a comparative multiparametric analysis of reactions of continuous HeLa-229 and RD cells and their nuclear and nucleolar apparatus to addition of solcoseryl to the culture medium.

MATERIALS AND METHODS

Cells (10^5/ml) were inoculated in 6-well plates with coverslips and cultured in Eagle's MEM with 10% bovine serum and glutamine. Solcoseryl (10 μg/ml) was added to some wells after attaining confluence. The slides were removed after 24 and 48 h in culture. The preparations were fixed in 96° ethanol and stained by Feulgen's method in our modification [5] for detection of DNA and with gallocyanin chrome alum [12] for detection of RNA, with methyl green pyronine [10] for detection of the nucleoli, with Mayer's hematoxylin

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and eosin [8] for cytomorphometry. DNA (λ=575 nm) and RNA (λ=634 nm) content was evaluated, the size of cells, nuclei, and nucleoli was estimated (at ×130) using an image analyzer designed on the basis of an SMP-05 scanning photometer microscope (Opton) using UPIAM-2000 software (Yu. A. Magakian, A. L. Kapantsyan, T. Yu. Magakian). A total of 100 cells were examined in each case. The monolayer density (number of cells per 0.01 mm^2 visual field), percentage of dead cells, proliferation activity (number of mitoses per 1000 cells), number of nucleoli in the nuclei, cell distribution in the population by the number of nucleoli in the nuclei, the nucleolar/nuclear proportion, cell RNA content, nuclear DNA and RNA content, nuclear volume and surface area, nucleolar summary volume and complete surface area, summary content of nucleolar DNA and RNA in the nucleus were evaluated. Before scanning the cell, nuclei, and nucleoli images were contoured with a marker. The data were processed using SPSS software [1].

RESULTS

Intact HeLa and RD cultures differ by the population parameters. The monolayer density in RD culture after 24 and 48 hours of culturing was 2-2.5 times higher than that of HeLa culture and, presumably, for this reason mitotic activity of cells in RD culture was much lower than in HeLa culture (Table 1). The cultures also differed by some other parameters: cellular and nuclear RNA content, nuclear volume and surface area (Table 2). The effects of solcoseryl on the population parameters of HeLa culture were as follows. The density of monolayer after 24-h culturing was 2-fold lower in intact culture; after 48 h it increased by almost 40%, but did not reach the density in intact culture, despite the fact that its values in intact culture somewhat decreased by this period (Table 1).

Proliferative activity of intact HeLa culture also decreased more than 2-fold after 48-h culturing. Addition of solcoseryl inhibited its activity still more: mitotic activity after 24 h was 2-fold and after 48 h 3-fold lower than in intact culture. Hence, in our experiment solcoseryl inhibited, but not stimulated cell proliferation (that is, exhibited activity opposite to the information provided by the manufacturer). Addition of solcoseryl resulted in a more than 2-fold increase in the count of dead cells in comparison with the control; this result was paralleled by general reduction of cell count in both groups (Table 1).

The effects of solcoseryl on HeLa cell parameters varied (Table 2). The drug virtually did not modify the content of RNA in cells and nuclei and the DNA content in the nuclei of these cells. The changes in these characteristics were negligible. The presence of solcoseryl in the medium had a negative impact for total DNA content in the nucleoli: these values, similar in the control and after 24-h culturing, decreased after 48 h in both groups, but just by 30% in the control and almost 2-fold in the culture with solcoseryl. Hence, solcoseryl stimulated this process. This was paralleled by a reduction of the total volume and surface area of nucleoli. In the presence of solcoseryl, these values were by 30% lower than in intact culture. The same was observed for nuclear volume and surface area. The only parameter which did increase was the total RNA content in nucleoli (Table 2). These data indicate that addition of solcoseryl to culture medium mainly inhibited the population and cellular parameters of HeLa culture.

The effect of solcoseryl on the RD culture population parameters were less pronounced: its addition to

TABLE 1. Population Parameters of Intact (Control) and Solcoseryl-Treated HeLa-229 and RD Cultures (*X*±*S*)

Parameter	Culture	Period of culturing for groups, h			
		24		48	
		intact (control)	solcoseryl	intact (control)	solcoseryl
Cell count per 0.01 mm^2	HeLa	9.5±0.6*	4.2±0.3*	8.7±0.3	7.5±0.5
	RD	20.1±0.7	21.2±0.3	19.5±0.7	19.5±0.9
Percentage of mitoses per 1000 cells, ‰	HeLa	3.20±0.01*	2.40±0.01*	1.40±0.06*	0.50±0.01*
	RD	0.90±0.05*	0.30±0.05*	0.80±0.04*	0.20±0.04*
Percentage of dead cells	HeLa	5.30±0.05*	11.70±0.02*	3.00±0.02*	6.30±0.06*
	RD	3.4±0.3	3.4±0.3	4.9±0.5	4.6±0.3

Note. Here and in Table 2: *p<0.05.

TABLE 2. Parameters of Cells, Nuclei, and Nucleoli in Intact and Solcoseryl-Treated HeLa-229 and RD Cultures ($X \pm S$)

Parameter	Culture	Period of culturing for groups, h			
		24		48	
		intact (control)	solcoseryl	intact (control)	solcoseryl
Cell RNA content, arb. units	HeLa	411.2±8.0	423.6±10.5	351.9±9.5	383.1±4.7
	RD	128.9±6.1	147.4±6.8	110.1±2.3	124.2±2.1
Nuclear DNA content, arb. units	HeLa	140.2±6.1	146.0±6.2	115.4±3.6	122.2±6.2
	RD	193.5±15.5	198.8±9.7	167.3±7.2*	297.3±7.2*
Nuclear RNA content, arb. units	HeLa	225.1±5.7	231.0±7.1	197.8±6.9	204.1±8.5
	RD	76.9±2.8*	103.6±2.5*	64.2±1.5*	83.7±1.8*
Nuclear volume, μ^3	HeLa	760.0±26.5*	648.0±19.0*	645.3±12.7*	581.0±14.9*
	RD	607.7±36.5	565.1±23.3	546.3±21.7	484.1±21.2
Nuclear surface area, μ^2	HeLa	175.9±3.7	168.5±3.7	164.1±2.2	155.5±3.5
	RD	152.8±4.3	159.7±4.2	133.8±3.3*	194.1±3.3*
Total nucleolar DNA content, arb. units	HeLa	14.3±1.0	14.2±1.1	9.4±0.8*	7.4±0.4*
	RD	15.8±0.8*	21.1±1.1*	18.1±0.8*	27.1±0.8*
Total nucleolar RNA content, arb. units	HeLa	21.2±1.4	22.8±1.2	16.9±1.7	17.9±1.1
	RD	19.3±1.1	21.9±1.2	14.5±0.7	15.7±0.8
Total nucleolar volume, μ^3	HeLa	22.7±1.6*	14.6±0.6*	15.6±0.6*	11.6±0.4*
	RD	11.8±0.7*	22.9±0.6*	16.4±0.6*	50.0±3.2*
Nucleolar complete surface, μ^2	HeLa	26.4±1.4*	17.9±0.7*	14.9±0.6*	10.3±1.3*
	RD	16.5±0.7*	29.9±0.8*	13.6±0.8*	22.0±6.2*
Nucleolar/nuclear ratio	HeLa	0.10	0.11	0.06	0.08
	RD	0.08	0.09	0.10	0.10
Mean number of nucleoli in nuclei	HeLa	2.20±0.05	2.31±0.07	2.10±0.06	2.02±0.04
	RD	1.90±0.01	1.71±0.01	2.20±0.02	2.20±0.02

culture medium virtually did not change monolayer density and percentage of dead cells, but significantly inhibited mitotic activity, which decreased 3-fold after 24 h and 4-fold after 48 h in comparison with the control (Table 1). The effect of solcoseryl on the cellular parameters of RD culture was more manifest. The cellular and nuclear RNA content increased (particularly after 48 h), as did the nuclear DNA content, nuclear surface area, nucleolar summary volume and summary complete surface area. The nucleolar/nuclear ratio and the mean number of nucleoli in the nuclei virtually did not change (Table 2).

The nucleoli (1-4) were detected in all cellular nuclei in both cultures, each nucleus containing 2 nucleoli, on average (Table 2). The presence and number of nucleoli in the nuclei is an important criterion characterizing the physiological status of the cells. Some authors [11,13] consider the nucleoli as the main indicators of cell activity. Distribution of the cells by the number of nucleoli in the nuclei in populations of both cultures was a dynamic parameter. This distribution was liable to change during cell culturing and under the effect of solcoseryl. The number of cells with mononucleolar nuclei decreased in HeLa population after 24 h of culturing in comparison with the control, while in RD culture the count of these cells increased. The number of cells with mononucleolar nuclei was the same (30%) in intact HeLa and RD cultures after 24 h and decreased after 48 h. In populations exposed to solcoseryl, the percentage of mononucleolar cells remained higher. Cells with binucleolar nuclei were most numerous in both cultures; the number of these

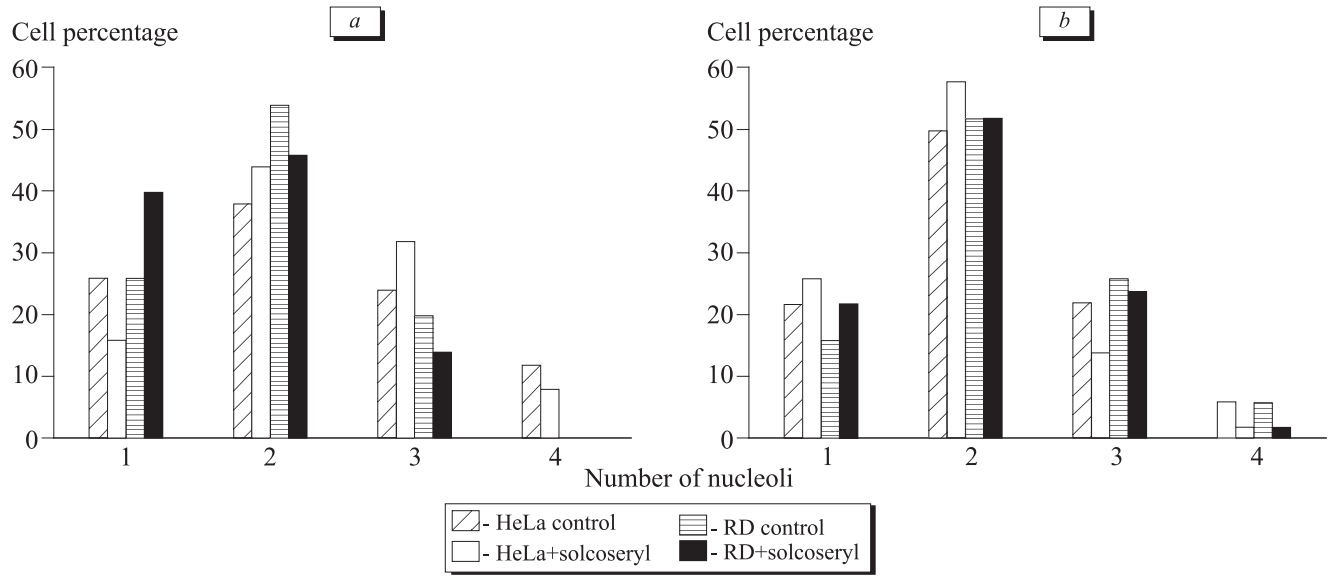

Fig. 1. Distribution of cells by the number of nucleoli in the nuclei and the effect of solcoseryl on this distribution.

cells increased in general during culturing. The percent of these cells in intact HeLa culture was 35% after 24-h culturing and 50% after 48 h. Solcoseryl treatment promoted an increase of their percentage in the population to 44% after 24 h and to 58% after 48 h of culturing. The percentage of cells with binucleolar nuclei in an intact RD culture was even higher and reached 55% after 24 h of culturing. After 48 h their share decreased to 50%, which was more than their percentage in a population of intact HeLa culture. Solcoseryl treatment led to a decrease in the percentage of cells with binucleolar nuclei to 45% after 24 h, while after 48 h their share increased again and reached the control level (Fig. 1).

The counts of cells with three nucleoli in the nuclei were significantly less in both populations than of cells with binucleolar nuclei. Solcoseryl treatment of HeLa cells led to an increase of their percentage after 24 h of culturing in comparison with the control, but after 48 h their share again decreased (almost 2-fold). The percentage of these cells in RD culture also decreased under the effect of solcoseryl. The least was the percent of cells with 4 nucleoli in the nuclei: 0% in an intact RD culture after 24-h culturing and just few cells after 48 h. The counts of cells with four-nucleolar nuclei in both intact cultures (RD and HeLa) were in general higher than in solcoseryl-treated cultures.

The decrease in the number of nucleoli in the nuclei was in line with reduction of the total content of DNA, volume and surface area of the nucleoli (Table 2). Addition of solcoseryl to cultures did not appreciably change DNA content in the nuclei, nuclear volume and surface area in comparison with the control.

Summing up our findings, we conclude that solcoseryl, added to culture media of HeLa and RD for 48 h, undoubtedly modified behavior of these cells at the population and cellular levels, its effects differing by intensity and direction. Hence, solcoseryl can be regarded as a bioactive compound with characteristics of a cell population growth regulation factor. Solcoseryl capacity to inhibit the proliferation and other vital activities of cells and cell populations by exhibiting the cytostatic characteristics is particularly interesting for biologists and physicians.

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