

The effect of a 10% solution of calcium chloride and its 6- and 12-hundredth homeopathic dilutions on the activity of apoptosis of large decidual cells

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on the apoptosis activity of large decidual cells

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SUMMARY

Terminally differentiated large decidual cells are heterogeneous in their DNA content. This is one of the signs of apoptosis. The activity of BDC apoptosis increases with the severity of the course of preeclampsia. It is also known that in the presence of calcium ions in the medium, the number of dead cells is greater than when cells are cultured in a medium that does not contain calcium ions. The DNA cytometric coefficient of variation of the G1 peak is an indicator of the number of cells at the initial stages of apoptosis. A clear dependence of the degree of change in the value of the coefficient of variation of the G1 peak when adding the 6th and 12th homeopathic dilutions of 10% calcium chloride solution to the initial level of apoptosis in the suspension of decidual cells was revealed.

Key words: apoptosis, large decidual cells, DNA cytometry, preeclampsia, homeopathic dilutions.

RESUME

Terminally differentiated large decidual cells are heterogeneous in their DNA expression. DNA expression is one of the signs of apoptosis. The activity of apoptosis of LDC increases as the course preeclampsia becomes heavier. It is also known that when calcium ions are present in the cells culture, the number of dead cells is greater than when cells culture does not contain calcium ions. The DNA cytometric coefficient of variation G1 peak is an indicator of the number of cells in the initial stages of apoptosis. We found a clear dependence of the degree of change in the value of the G1 peak coefficient of

variation when adding 10% calcium chloride solution to cells culture of the 6c and 12c homeopathic dilutions on the initial level of apoptosis in the suspension of decidual cells was revealed.

Keywords: apoptosis, large decidual cells, DNA cytometry, preeclampsia, homeopathic dilutions.

INTRODUCTION

It is known that terminally differentiated large decidual cells (BDC) are heterogeneous in their DNA content [1]. It is also known that this is one of the signs that the death of these cells occurs by the type of programmed cell death or apoptosis [2, 3]. Apoptosis has recently been given great importance in maintaining cell homeostasis [4, 5, 6]. In particular, the importance of apoptosis in atrophy of the endometrium of women during the menstrual cycle has been proven [7]. Our previous works have shown an increase in the activity of BDC apoptosis as the course of preeclampsia worsens and the ability to judge the level of their apoptosis by DNA cytometry data [8].

When working out the technique of DNA cytometry of decidual membrane cells, we found that in the presence of calcium ions in the medium, the number of dead cells is greater than when cells are cultured in a medium that does not contain calcium ions (Versene solution). In this regard, it was decided to study the effect of a 10% solution of calcium chloride and its 6- and 12-hundredth homeopathic dilutions on the level of apoptosis of decidua cells.

MATERIALS AND METHODS

Fragments of the decidua of 55 puerperas from 19 to 35 years old were examined. The average age was 25.8 ± 1.3 years. In 21 women, pregnancy and childbirth proceeded physiologically. In 23 patients, pregnancy and childbirth were complicated by mild preeclampsia. Severe preeclampsia was diagnosed in 11 patients. All deliveries occurred between 38 and 41 weeks of gestation.

The decidua for research was taken from three places near the placenta and two in the area of rupture of the membranes. The decidua was separated from the chorion mechanically, the completeness of the separation was controlled histologically. After mechanical grinding, the pieces of tissue were placed in a Versene solution with the addition of collagenase (1 mg / ml, produced by the Pacific Institute of Bioorganic Chemistry, Russian Academy of Sciences). The incubation was carried out in a thermostat at 37 ° C for 45 min. After filtration through nylon filters, the cell suspension was centrifuged at 400 g for 10 min, followed by resuspension in Versene's solution. The suspension for experiments contained from 500,000 to 2,000,000 cells per ml.

In a series of experiments with calcium chloride, a 10% solution of calcium chloride was used (an ampouled preparation used for intravenous injections). It was also used to make 6- and 12-hundredth homeopathic dilutions. For this, the initial and subsequent solutions were successively diluted in a ratio of 1: 100, shaking each newly obtained dilution at least 50 times, as is customary in homeopathy [9, 10]. In the first case, 6 consecutive dilutions were carried out,

in the second - 12. For the manufacture of homeopathic dilutions used bidistilled water.

A suspension of decidua cells was distributed into 5 test tubes, 2 ml each. One tube was used as a control. In the remaining 4 test tubes, 40 microliters of one of the solutions were added: 10% calcium chloride solution, the 6th centesimal dilution of this solution, its 12th centesimal dilution, and pure bidistilled water. Then all tubes were incubated at 37 ° C for 30 min.

Next, DNA cytometry was performed according to the previously described methods [11, 12]. As a result of mathematical processing of DNA histograms, the coefficient of variation of the G1 peak was determined as an indicator reflecting the degree of heterogeneity of the cell population in terms of the DNA content in cells with a normal (diploid) set of chromosomes and, therefore, the number of cells in the studied population at the initial stages of programmed cell death.

RESULTS

Analysis of the data obtained showed that the level of apoptosis of decidual membrane cells increases sharply when a 10% solution of calcium chloride (20 µl per 1 ml) is introduced into the medium. Despite the wide range of values of the coefficient of variation of the G1 peak in this group of experiments (5.26 ± 0.68), the studied indicator in it was statistically significantly higher ($p < 0.009$) than in the control group (3.53 ± 0.23) ...

When studying the effect of homeopathic dilutions of 10% calcium chloride solution on the activity of programmed cell death in a suspension of decidual cells, the lowest values of the coefficient of variation of the G1 peak were found using the 6th centesimal dilution (3.07 ± 0.24). When using the 12th centesimal dilution of 10% calcium chloride solution, the level of variation coefficient G1 of the peak was slightly higher (3.17 ± 0.19). However, no statistically significant difference between the values of the studied parameter in these groups and in the control group was found. We hypothesized that this may be due to the pronounced dependence of the degree of decrease in the level of apoptosis under the influence of homeopathic dilutions of a 10% solution of calcium chloride on the initial level of apoptosis in a suspension of decidual cells.

The correlation coefficient between the initial values of the G1 variation coefficient of the peak and the degree of its change when the 12th centesimal dilution of 10% calcium chloride solution was added to the medium was -0.741. When comparing the values of the coefficient of variation of the G1 peak in the control group of experiments and the group of experiments with the 6th centesimal dilution of 10% calcium chloride solution, the correlation coefficient between them turned out to be -0.931. This unambiguously testifies to the dependence of the degree of change in the value of the coefficient of variation of the G1 peak when adding homeopathic dilutions of 10% calcium chloride solution to the medium on the initial level of apoptosis in the suspension of decidual cells. Indirect confirmation of the identified

The regularity is a high positive correlation between changes in the values of the coefficient of variation of the G1 peak in the groups of experiments with 6th and 12th centesimal dilutions of 10% calcium chloride solution. The correlation coefficient between them is 0.904.

A similar pattern was not found when comparing the values of the coefficient of variation of the G1 peak in the control group of experiments and in the group of experiments with a 10% solution of calcium chloride. The correlation coefficient between the studied parameters in these groups was equal to 0.423, which indicates that there is no relationship between the initial level of apoptosis and the degree of its change under the influence of 10% calcium chloride solution.

It should be noted that after the addition of pure bidistilled water, a slight decrease in the values of the coefficient of variation of the G1 peak was also noted (3.38 ± 0.14). However, neither a statistically significant difference, nor any correlations with the values of the coefficient of variation G1 of the peak in the control group of experiments in the group of experiments with pure bidistilled water could not be identified. As there were no correlations between the values of the coefficient of variation of the G1 peak in the described group of experiments and experiments with homeopathic dilutions of 10% calcium chloride solution.

DISCUSSION AND CONCLUSIONS

Thus, summarizing the obtained data, we can note that on the model we have chosen, the homeopathic postulate about the reverse effect on biological systems of standard chemical and homeopathic solutions of initial substances is confirmed. Which confirms the famous statement of Paracelsus: "Medicine is poison, but poison is medicine. A single dose will transform medicine into poison and poison into medicine ... "[13]. And, to a certain extent, the basic rule of homeopathy: like is treated like [14].

On the other hand, this model clearly demonstrated the complexity of tracking the results of the impact on biological systems of homeopathic drugs, in particular, the dependence of the degree of effectiveness of the latter on the initial parameters of the target and the correctness of the choice of homeopathic dilution. The pronounced "individualization" of the effect of homeopathic preparations significantly complicates the construction of the study design and the mathematical processing of the results.

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