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GLYCOPEPTIDE VACCINE ON DNA-HISTONE CARRIER AND ITS IMPACT ON Z-POTENTIAL OF EFFECTOR CELLS DURING EXPERIMENTAL TREATMENT OF LYMPHOBLASTIC LEUKEMIA

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Aim: **To increase the effectiveness of anticancer vaccine delivery and activity via its immobilization on reconstructed chromatin (RC).** ***Materials and Methods:*** **The hybrid mice of CDF-1 line with transplanted P-388 leukemia were used. Reconstructed chromatin was obtained from the thymus of inbred rats. Glycopeptide cancer vaccine prepared from P-388 leukemia cells, was immobilized of RC. ζ-potential of thymocytes from tumor-bearing mice was calculated from Smoluchowski equation.** ***Results:*** **The data have demonstrated the principal possibility of using the fragments of reconstructed chromatin of heterologous origin as a carrier of glycopeptide cancer vaccine prepared from the cells of P-388 leukemia. Preventive immunization with the correspondingly immobilized vaccine normalized ζ-potential of thymocytes in animals with transplanted P-388 leukemia.** ***Conclusions:*** **The use of immobilization of glycopeptide cancer vaccine on reconstructed chromatin improves anticancer activity of the vaccination.**
Key Words: **glycopeptide cancer vaccine, reconstructed chromatin, ultrasonic disintegration, ζ-potential.**

Our previous studies showed that fragments of reconstructed chromatin (RC) obtained by ultrasonic disintegration can inhibit the growth of transplanted tumor models, depending on the origin of chromatin. In particular, RC from the thymus of rats reliably inhibited the growth of mouse sarcoma Sa-37. However, RC of homologous origin, isolated directly from B-16 melanoma cells, caused a strong stimulation of tumor growth [1].

In the case of pronounced antitumor action, RC of heterologous origin demonstrated efficacy, which can be validly compared with the efficacy of the earlier developed glycopeptide cancer vaccines (GCV) [1–3].

This property of RC gave a theoretical basis for the schemes of its use in conjunction with GCV, when chromatin and vaccine injections were made in parallel, without mixing. However, a reliable potentiation of the action of cancer vaccine has not been identified [4]. The use of a mechanical mixture of RC and GCV did not affect the vaccine therapeutic properties either.

Nuclear chromatin is known to dissociate better in solvents with higher ionic strength. This property was used in the proposed method of immobilization of glycopeptide vaccines on fragments of RC. RC, which has the inherent ability to be actively captured by living cells, is suggested to cause not only their apoptotic destruction [1, 4, 5], but, in parallel, as a very specific cancer vaccine carrier, to increase the effect of vaccine on the formation of the immune response of a body afflicted with a malignant process.

In isotonic NaCl RC forms sols, and in hypertonic, for example, 2.0 M NaCl it gives true solutions primarily due to specific unwinding of double helix of DNA-histone complex. This leads to RC absorption spectrum with more characteristic for nucleic compounds profile with a pronounced minimum at 230 nm and the peak corresponding to 260 nm, compared with a smoothed curve of its absorption in isotonic medium (Fig. 1) [1].

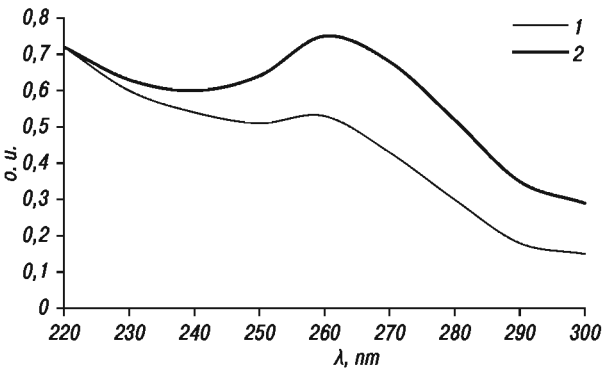

Fig. 1. Absorption spectra of solutions of ultrasound-disintegrated RC fragments from rat thymus (0.1 μl/ml) prepared in 0.14 M NaCl (1) and 2.0 M NaCl (2)

The novelty of the method described, unlike the preparation of a mechanical mixture of GCV and RC, is that previously dissolved in a small volume of isotonic NaCl cancer vaccine is brought into contact with significantly larger volume of reconstructed with ultrasonic disintegration chromatin solution prepared in 2.0 M NaCl 20 h in advance.

More complete than when mixed in isotonic medium contact of GCV molecules with the untwisted in hypertonic NaCl molecules of RC occurs at 37 °C for 1 hour.

Subsequently, necessary amount of distilled water is added to the solution of GCV, already adsorbed to a significant degree on nucleoprotein carrier, to form isotonic dispersion medium. The resulting mixture is placed in a refrigerator, where colloidal precipitate of GCV immobilized on RC forms at 4 °C during a night.

MATERIALS AND METHODS

We used the animals bred in IEPOR NASU vivarium: inbred rats and hybrid mice of CDF-1 line. The animals were kept under standard conditions, had free access

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Abbreviations used: GCV – glycopeptide cancer vaccines; RC – reconstructed chromatin; remosomes – reconstructed chromatin.

to food and water and were derived from the experiment with the standards of biological ethics.

RC was obtained from the thymus of a young mongrel female rat with body weight of 150 g, as described in [1, 4].

GCV was prepared according to standard procedures [2, 3] from the tumor cells of P-388 leukemia on the fifth day after inoculation of ascitic cells into mice of CDF-1 hybrid line. Further, at the initial samples, RC, and GCV were stored under ethanol at –12 °C.

An aliquot of resuspended in alcohol GCV precipitate, corresponding to an equivalent of 1×10^7 of tumor cells, was precipitated from ethanol by short-term centrifugation (3000 rpm×2 min) and dissolved at vigorous stirring in 0.5 ml of 0.14 M NaCl, after which the solution was left overnight at 4 °C. In parallel, 30 µl of RC gel precipitate were dissolved in 5 ml of 2.0 M NaCl and also left overnight at 4 °C.

The next day, GCV and RC solutions were kept at 20 °C for 1 h, then 0.5 ml of GCV solution in 0.14 M NaCl and 4.5 ml of RC solution in 2.0 M NaCl were mixed at vigorous stirring and placed into a thermostat at 37 °C.

One hour later the aliquot (1 ml) of the mixture of GCV and RC solutions was sampled, and then the remaining mixture was frozen at –12°C for further storage. Distilled water in amount of 11 ml was added to the aliquot to get 12 ml of 0.14 M NaCl solution, containing 2×10^6 cell. eq. of GCV and 6.0 µl of RC starting material simultaneously.

The solution was left in a glass centrifuge tube for one more night at 4 °C. Within 20 h loose sediment formed on the bottom of the tube, which is suggested to be the GCV immobilized on the RC (remosoms).

During *in vivo* experiments animals of CDF-1 line were preventively vaccinated with auto-GPV immobilized on heterologous RC at a concentration corresponding to the absorption $E_{260} = 0.65$ (Fig. 2).

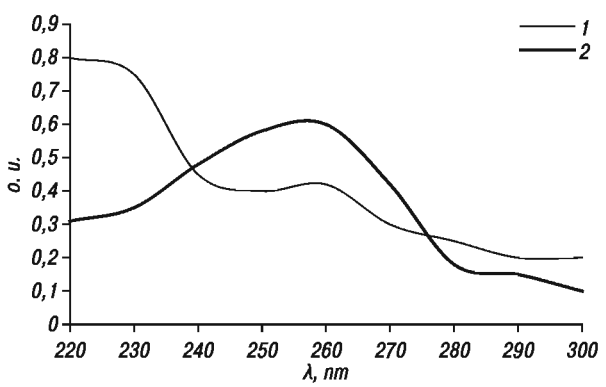

Fig. 2. Absorption spectra of supernatant (1) and colloidal solution of GCV on DNA-histone carrier (2) from the precipitate formed in 0.14 M NaCl

Colloidal solution of vaccine on DNA-histone carrier in 0.2 ml of isotonic NaCl was subcutaneously injected into the lumbar region of animals three times, at intervals of 48 and 24 h. A day after the last vaccination, P-388 leukemia was transplanted into the abdominal cavity of mice in amount corresponding to 3×10^7 cells in 0.2 ml of 0.9% NaCl. Both control and experimental animals were killed on the sixth day after inoculation; their inguinal lymph nodes, thymus and spleen were removed for further study. Lymphocytes were obtained by mechanical disintegration of the relevant organs with further washing of lymphoid cells by centrifugation in a special buffer for cell electrophoresis, as described in [6]; the electrophoresis itself was performed according to the method described in [6, 7].

ζ-potential was calculated from Smoluchowski equation. The value of linear cell velocity in electric field was inserted in the equation. This velocity was measured using a device in which cells, suspended in a special K/Na-phosphate buffer, moved inside a flat-wall quartz capillary. This eliminated extraneous influences on the mechanical nature of cell movement and made possible the use of microvolumes of the material [8, 9].

The distance between the platinum electrodes in the device was 5 cm, and the gradient of the electric field intensity E reached 20 V/cm, as the voltage applied to the electrodes was 100 V. Smoluchowski equation, adapted to biological and, automatically, to the physiological values of physicochemical parameters is given as:

$$\zeta = 14U,$$

where U is the electrophoretic mobility, expressed in miscellaneous units as the ratio of the linear cell velocity in the electric field, determined experimentally (µm/s), to the gradient of the field intensity (V/cm) [8].

The direction of cell movement under the influence of the external electric field with specific parameters (towards the cathode or the anode) made it possible to determine the sign of the total cell charge, and its surface density (q) was calculated according to the Quincke — Helmholtz equation:

$$q\delta = \zeta\varepsilon_a,$$

where δ is the thickness of the electric double layer, and ε_a is the absolute dielectric constant [10].

RESULTS AND DISCUSSION

Fig. 3 shows the absorption spectrum of GCV solution with concentration equal to the concentration used for immobilization, namely 1.67×10^5 eq/ml. This Figure also shows the absorption spectrum of the RC in an appropriate concentration (0.5 µl of starting material in 1 ml of 0.14 M NaCl).

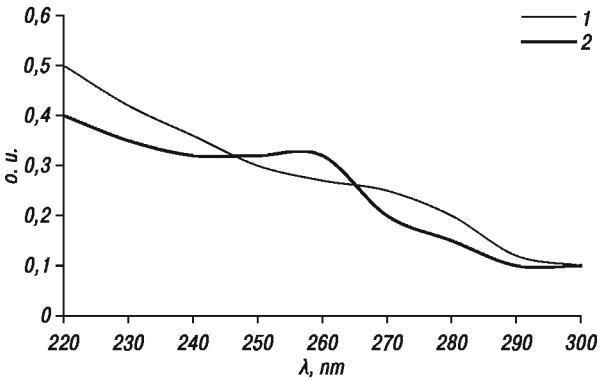

Fig. 3. Absorption spectra of GCV from P-388 tumor cells (1) and ultrasound-disintegrated RC fragments from rat thymus (2) in 0.14 M NaCl in concentrations of 1.67×10^5 cell/eq/ml and 0.5 µl/ml respectively.

The absorption spectra of the supernatant of nonagitated mixture and colloidal solution of the precipitate formed during a night in the cold, were plotted separately. For this purpose, the precipitate was diluted after decantation in a volume of isotonic NaCl equal to the volume of supernatant fraction (see Fig. 2).

It should be noted that the geometric addition of the absorption curves of vaccine and RC solutions in concentrations used for immobilization (see Fig. 3), does not give the absorption spectrum of the supernatant fraction of the mixture after immobilization process (see Fig. 2). It is in the colloidal precipitate that the material absorbing at the interval of 240–270 nm was observed. This gives grounds to state with a sufficiently high degree of probability that the precipitate formed in the cold in the last stage of GCV/RC interaction is the glycopeptide cancer vaccine immobilized on fragments of RC or remosoms.

The significant difference of the method described is that immobilization of glycopeptide cancer vaccine on RC was proposed to take place in hypertonic dispersion medium (2.0 M NaCl) where the untwisting of the double helix of DNA-histone complex makes the negatively charged nucleic acid more accessible for sorption of positively charged carbohydrates and polypeptide chains of vaccine. Further recovery of double-helical structure of the compound in isotonic dispersion medium probably contributes to the firm holding of GCV molecules on the DNA-histone carrier.

In vivo experiments showed that the development of P-388 leukemia within six days after inoculation caused nonsignificant reduction in ζ-potential of mature lymphocytes from peripheral lymph nodes at physiological pH values (7.4). Compared to control values (9.43±0.53 mV), lymphocytes of tumor-bearing mice had electrokinetic potential equal to 7.42±0.80 mV. However, lymphocytes of thymus and spleen mononuclear cells responded to tumor growth with reliable, significantly larger reduction of ζ-potential at pH 7.4 — on 37.5% for thymocytes and 30.3% for splenocytes (Table 1).

Further, the effect of tumor growth on ζ-potential and total surface charge density of lymphoid cells at neutral pH of the dispersion medium was investigated. The reasons for this were, in the first place, known in the literature resistance of many tumor cell to high acidity, which causes possible contact of effector cells with target cells precisely at pH values close to 7.0, as well as the fact that, according to Jensen, living cells in neutral pH range have something like "isoelectric point", in which the total surface charge, negative at physiological conditions of the environment, changes to positive [8]. That is why the measurements at pH 7.0 make it possible to identify some subtle influence of external factors, hidden at physiological pH of 7.2–7.4.

The first thing that catches your eye is positive charge of the cells studied, unlike that at pH 7.4. Tumor growth reduced the negative cell charge at pH 7.4, while the positive charge of mature lymphocytes and spleen mononuclear cells at pH 7.0 revealed a statistically significant upward trend. It is noteworthy that thymocytes at neutral pH showed almost no reaction on the development of lymphocytic leukemia; neither their electrokinetic potential nor total positive charge in terms of area unit of cell surface changed (Tables 1, 2). It may indicate an expression of positively charged molecular hydrophilic groups on the surface of lymphocytes in weakly alkaline medium, perhaps those of transmembrane pores of protein nature. It is also possible that at pH 7.0, in response to the growth of lymphocytic leukemia ascites, new negatively charged elements express on the surface of mature lymphocytes from peripheral lymph nodes and spleen lymphocytes, otherwise the charge of hydrophilic groups mentioned above reverse. The fact that immature cells from thymus responded to tumor growth in different way as mature lymphocytes and splenocytes (see Table 2) deserves close attention.

Vaccination in a preventive mode caused almost normalization of ζ-potential of thymocytes, had very small effect on spleen lymphocytes and practically did not change the electrokinetic potential of mature lymphocytes from peripheral lymph nodes (Table 3). This is well matches the results of our previous studies, according to which the prior targets for GCV action are the cells of thymus not yet fully acquired the immune competence (unpublished data).

In conclusions preventive vaccination with auto-GPV immobilized on remosoms promoted the recovery of ζ-potential of thymocytes in animals with inoculated P-388 tumor to the intact control level. The target cells for the action of GCV on DNA histone carrier, as for GCV, taken alone, are thymic lymphocytes, has not yet acquired immune competence.

Table 1. Impact of medium pH on the electrokinetic potential (ζ, mV) of lymphocytes of intact and P-388-bearing mice

Object	Lymphocytes from					
	peripheral lymph nodes		thymus		spleen	
	pH 7.0	pH 7.4	pH 7.0	pH 7.4	pH 7.0	pH 7.4
Intact CDF-1 mice	7.35 ± 1.10	9.43 ± 0.52	12.56 ± 1.74	14.5 ± 1.2	11.25 ± 1.24	12.62 ± 0.94
P-388-bearing CDF-1 mice	8.74 ± 0.85	7.42 ± 0.80	12.25 ± 1.15	9.06 ± 1.0	13.15 ± 0.95	8.80 ± 0.50

Table 2. Impact of medium pH on total surface charge density (q, x 10^{-2} C/m^2) of lymphocytes of different origin from intact and P-388-bearing mice

Object	Lymphocytes from					
	peripheral lymph nodes		thymus		spleen	
	pH 7.0	pH 7.4	pH 7.0	pH 7.4	pH 7.0	pH 7.4
Intact CDF-1 mice	+5.27 ± 0.8	-6.76 ± 0.37	+9.01 ± 1.25	-10.4 ± 0.9	+8.1 ± 0.89	-9.05 ± 0.68
P-388-bearing mice	+6.27 ± 0.6	-5.32 ± 0.57	+8.78 ± 0.82	-6.5 ± 0.7	+9.42 ± 0.7	-6.31 ± 0.36

Table 3. The effect of prophylactic vaccination with auto-GCV on DNA-histone carrier (auto-GCV/RC) on the values of the electrokinetic potential (ζ, mV) of lymphocytes of different origin at physiological pH range (7.4)

Object	Lymphocytes from		
	peripheral lymph nodes	thymus	spleen
Intact control	9.43 ± 0.52	14.5 ±1.2	12.62 ± 0.94
Untreated P-388-beaimg mice	7.42 ± 0.80	9.06 ± 1.0	8.80 ± 0.52
Course of GPV/RC before the inoculation of P-388	7.64 ± 0.92	13.4 ± 0.84	9.86 ± 0.52

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