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Interaction of tumor and normal blood cells with ethylene oxide and propylene oxide block copolymers

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Abstract Ethylene oxide and propylene oxide block copolymers (pluronics) are widely known as agents that promote drug penetration across biological barriers. We have studied the interaction of normal and malignant blood cells with pluronics L61 and P85 that have different hydrophobicity. SP2/0 myeloma cells accumulated pluronics while normal cells adsorb most of the polymer on the surface. Interaction of pluronics with cells resulted in drastic changes of membrane microviscosity. Tumor cell membrane microviscosity decreased after pluronics adsorption, in contrast to normal cells, whose membrane microviscosity was enhanced. We suppose that sensitivity of tumor cell membrane microviscosity to the pluronics action correlates with its permeability for molecular substances.

Key words: Pluronic; Myeloma; Lymphocyte; Erythrocyte; Binding; Microviscosity

1. Introduction

Ethylene oxide and propylene oxide block copolymers are widely used in pharmacology as components of artificial blood [1–3], vaccines [4], agents for drug solubilization [5,6] and thermotropic gels for drug delivery [7,8]. In a number of papers, pluronics were reported as promoting drug penetration across different biological membranes (e.g. cell membrane [9,10], the blood-brain barrier [11], and skin [12,13]). Recently it has been shown that pluronics L61 and P85 dramatically enhance the sensitivity of gP-170 containing multi-drug resistant cells to anthracycline antibiotics [14,15]. A combined application of anti-tumor drugs with pluronics resulted in the accumulation of chemotherapeutics in tumor cells and decreased the side effects of drugs on the immune system [16]. The reasons for these phenomena remain unclear.

The biological effects of pluronics are possibly mediated by their interaction with cell plasma membranes and the modulation of their permeability. It is well known that polyethylene oxide containing polymers can affect biological membrane structure. Pure polyethylene oxide (PEO) at 5% concentration and more induces changes in the lipid bilayer hydration sphere and causes membrane fusion [17]. Lower PEO concentrations (0.1–1%) induce changes in membrane fluidity [18]. PEO containing detergents such as Triton X-100 bind the biological membranes so that their hydrophobic groups incorporate into the lipid bilayer, thus changing the membrane structure and fluidity [19]. Ethylene oxide (EO) and propylene oxide (PO) block copolymers can also interact with lipid bilayers, changing the thermodynamic properties of the latter [20]. In the present work we have investigated the interaction of two pluronic copolymers with membranes of different normal and malignant cells, and their influence on the membrane fluidity.

2. Materials and methods

2.1. Cells

Mouse splenocytes were isolated from mouse spleen as described in [21]. Briefly, spleens of 6–8 months old BALB/c mice were washed thoroughly in 5–10 ml of saline to remove contaminating blood and carefully pounded in 2–3 ml DMEM medium. The powder was filtered through gauze and centrifuged at 300–500×*g* to gather splenocytes. The cells were used immediately after isolation.

Erythrocytes were isolated from blood of BALB/c mice supplemented with EDTA. The cells were washed three times with DMEM and used immediately. Mouse myeloma cells SP2/0 were cultured in RPMI 1640 medium in the presence of 10% fetal calf serum, 0.075% sodium bicarbonate and 2 mM glutamine. The cells were propagated as ascites tumors in BALB/c mice by intraperitoneal injection of 5–8 million cells per mouse. Ascites fluids were collected 3–4 weeks after injection and the cells were gathered by centrifugation, washed three times with saline and used immediately after isolation.

2.2. Tritium labelled pluronics

The radioactive label was introduced into the pluronic molecule by treatment of a thin pluronic film with atomic tritium according to [22]. Briefly, 2 mg of pluronic L61 $(EO)_2(PO)_{30}(EO)_2$ or P85 $(EO)_{25}(PO)_{40}(EO)_{25}$ (Serva) dissolved in 2 ml of methanol was spread over reaction bulb walls and the solvent was evaporated under reduced pressure. 0.01 Pa pressure has been achieved, tritium was injected into the apparatus up to 0.5 Pa. The tritium-protium exchange reaction was initiated by a short (10 s) heating impulse of a tungsten helix at 2000°C. After two 10 s heating impulses, the unincorporated tritium was removed under vacuum and the procedure was repeated with a new portion of tritium. Then the pluronic film was dissolved in methanol and the solution was incubated for several days to remove exchangeable tritium atoms. The solvent was evaporated under vacuum and the dissolution-evaporation procedure was repeated three times. The residual material was dissolved in ethanol and purified from products of the polymer decomposition by gel filtration on a Sephadex LH-20 column (1.1×25) using ethanol as an effluent. The retention volume of the first peak containing most radioactive material corresponded to the molecular weight of intact pluronic. It was collected and the solvent was evaporated under vacuo. Pluronic final concentration was detected using the BaI_2 assay [23]. The specific radioactivity of the labelled polymer depended on the film thickness and labelling conditions and varied in most cases from 0.5–3 Ci/mmol.

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Abbreviations: DPH, 1,6-diphenyl-1,3,5-hexatriene; PPO, polypropylene oxide; PEO, polyethylene oxide; PO, propylene oxide; EO, ethylene oxide

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2.3. Binding of ^{3}H-labelled pluronics with cells

30–60×10^6 cells were suspended in 1 ml of DMEM, containing 20 mM HEPES, kept 30 min at the appropriate temperature (4°C or 37°C) and incubated with ^{3}H-pluronics for 120 min at the same temperature. The final concentration of pluronic varied in 0.2–2 μM intervals. This range of concentrations corresponds to the polymer concentration in blood achieved during treatment with pluronic-based formulas [16]. After incubation the cells were washed five times with cold PBS by centrifugation at 500×*g* and dissolved in 100 μl of 1 M NaOH. 25 μl of the solubilized material were used for determination of protein concentration according to Lowry [24] and 75 μl were neutralized with 225 μl of 0.4 N HCl and mixed with 3 ml of Triton X-100 containing toluene scintillation solution. Radioactivity was then measured using a Delta-400 scintillation counter.

2.4. Cell membrane microviscosity

Cell membrane microviscosity was measured using fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) (Reanal) according to Shinitzky and Barneholtz [25] and Plasek and Jarolim [26]. Briefly, 0.2 mM DPH in acetone was diluted 100-fold with Hanks' solution and the organic solvent was removed by the bubbling of the solution with nitrogen for 90–120 min. The cell suspension in Hanks' solution (10^6 cells/ml) was mixed with an equal volume of the labelling solution and stirred for 60 min at room temperature. Incorporation of DPH into the cell membrane results in a 10-fold increase of its fluorescence [25,26]. The suspension of labelled cells was mixed with pluronic solutions and incubated at 37°C for 1 h under gentle stirring. Fluorescence anisotropy was then measured using a polarization spectrofluorometer Hitachi F-4000, using an excitation wavelength 366 nm and an emission wavelength 433 nm. Microviscosity of the membrane hydrophobic area was calculated from the Perrin equation, r_o being 0.368, τ being 10 ns and $C(r)$ being 8.6×10^5 poise s^{-1} K^{-1} respectively as they were previously reported [25,27]

2.5. Isolation of mouse liver microsomes [28]

Liver (2 g) of an adult BALB/c mouse was homogenized in 6 ml 10 mM MOPS, pH 7.0 in a Polytron homogenizer (Kinematika, Sweden) at power 4 (2 impulses each of 30 s) in an ice bath. The sample was centrifuged at 600×*g* in an Eppendorf centrifuge for 10 min. The pellet, containing undisrupted cells and nuclei, was discarded and supernatant was centrifuged at 46 000×*g* using Beckman J2–21 centrifuge with an 18.1 rotor. The pellet was resuspended in 6 ml of 10 mM MOPS buffer, pH 7.0 and centrifuged again at 46 000×*g* for 60 min. Analysis of membrane marker enzymes showed that the microsomes preparation contained equal quantities of plasma membranes and membranes of endoplasmic reticulum.

2.6. Small unilamellar liposomes labelled with DPH [29]

Small unilamellar liposomes were prepared from egg yolk phosphatidylcholine (Sigma) or the mixtures of phosphatidylcholine with cholesterol or phosphatidylserine (Sigma). 10 mg of lipids in ethanol was mixed with 10 nmol of DPH and dried under reduced pressure. The material was suspended in 1 ml of PBS with vigorous shaking and sonicated (frequency 22 kHz, 2×200 s) using an ultrasound generator 4710 (Cole-Palmer Instruments). The obtained suspension of vesicles was centrifuged at 12 000 rpm on an Eppendorf centrifuge to remove large particles. The mean hydrodynamic diameter of the liposomes determined by quasi-elastic light scattering was proved to be 50–65 nm.

2.7. Determination of average cell size

The cells were placed in a hemocytometer and microphotographs at phase contrast (1×400) were taken. Cell diameter was measured and calculated in relation to the 50 μm side of the small square in the hemocytometer. The mean cell diameter was determined as an average of at least 20 cells.

3. Results

The ability of pluronic copolymers to promote drug penetration across biological barriers may result from their binding with cell membranes. To elucidate this point we investigated the binding of two copolymers P85 and L61, differing in PPO/PEO ratio, with normal and tumor blood cells. Pluronic

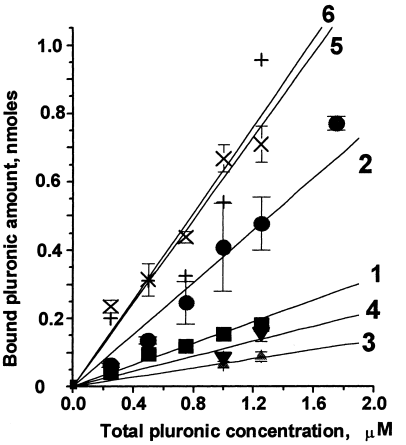

Fig. 1. Binding of ^{3}H-labelled pluronics with lymphocytes (curves 1, 2), erythrocytes (curves 3, 4) and myeloma cells SP2/0 (curves 5, 6) at 4°C in DMEM medium. Curves 1, 3, 5 pluronic P85; curves 2, 4, 6 pluronic L61.

concentration was varied in the 0.2–2 μM range, which corresponded to its concentration in the blood of animals treated with pluronic-based formulas. In preliminary experiments we showed that at 4°C, when an active uptake of pluronic is mainly inhibited [30], the binding reaction reached equilibrium after a 2 h incubation. It turned out that the binding of both pluronics with erythrocytes, splenocytes and myeloma cells was described by nearly linear binding isotherms (Fig. 1) which obviously indicates a non-specific character of pluronic binding with cells. This conclusion was confirmed by the fact that bound ^{3}H-labelled pluronic was not substituted by a 100-fold excess of unlabelled copolymer.

The efficiency of P85 and L61 pluronics binding with different cells depended on the cell type, temperature and pluronic hydrophobicity (Fig. 2). Binding data are presented on this figure as a number of the copolymer molecules per 1 μm^2 of cell surface. Comparison of binding efficiency at 4°C when endocytosis should be inhibited gives information about the influence of cell surface properties on the interaction with pluronic. Obviously, erythrocytes are characterized with a much lower binding efficiency with both pluronics as compared to lymphocytes and myeloma cells. Myeloma cells bind pluronic somewhat less effectively than splenocytes.

Binding efficiency depends slightly upon pluronic hydrophobicity. The more hydrophobic L61 pluronic (HLB = 3) bound to the cells 1.5–2.5 times more effectively than the water-soluble pluronic P85 (HLB = 16). These data are in a good agreement with the previously published observation that the therapeutic effect of the hydrophobic PPO-rich L61 is higher than that of the more hydrophillic PEO-rich P85 pluronic [14–16].

The character of the copolymers binding with myeloma cells and splenocytes was drastically changed at normal temperature. At 37°C a persistent accumulation of pluronic in the cells occurred (data not shown), which was presumably due to the fluid-phase endocytic uptake of the polymer adsorbed on the cell surface. The amount of the copolymer accumulated in cells can be estimated from the data of Fig. 2a,b as the difference between the amount of pluronic bound at 37°C and 4°C. The results of such calculation are presented on Fig. 2c. Myeloma cells accumulated 3.5 times more copolymers as com-

pared to normal splenocytes. Erythrocytes did not accumulate pluronics at all.

These results permit us to suggest that the physiological effects of pluronics in many cases can be mediated by their adsorption on cell membranes. We applied the method of fluorescence polarization to investigate changes in the bilayer structure caused by pluronic binding. This method was proposed more than 20 years ago and is based on the monitoring of the fluorescence depolarization, which depends on the rate of rotation of a fluorescent probe incorporated into the membrane. In our work we used DPH which nowadays is the most widely used probe for the measuring of membrane microviscosity.

The Perrin equation gives a link between the polarization anisotropy r and the viscosity of the probe microenvironment in the bilayer (η) [25]:

$$\frac{r_o}{r} = 1 + C(r)\frac{T\cdot\tau}{\eta} \quad (1)$$

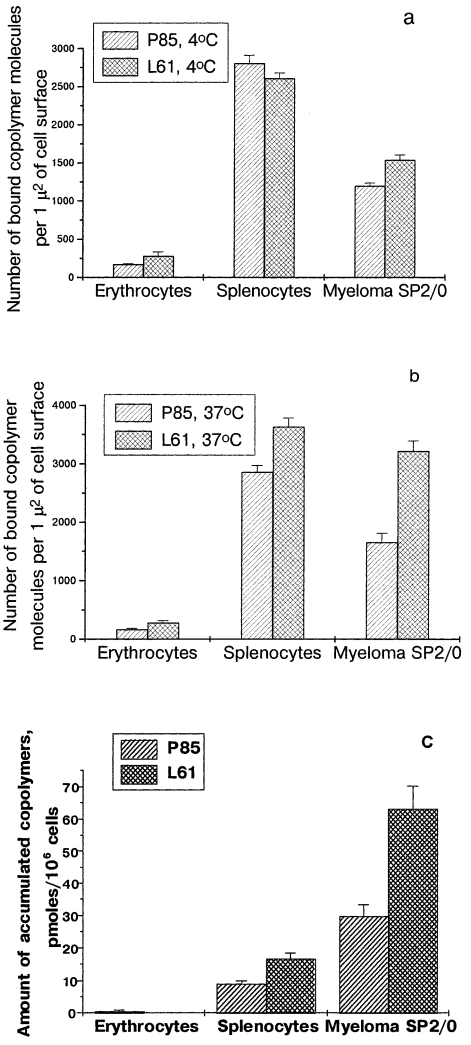

Fig. 2. Number of bound P85 and L61 copolymers chains per 1 μm^2 of cell surface after incubation with 1 μM copolymer solution for 2 h at 4°C (a) and at 37°C (b). c shows the amount of P85 and L61 copolymers accumulated in 10^6 different cells calculated as a difference between amount of the copolymer bound at 37°C and at 4°C.

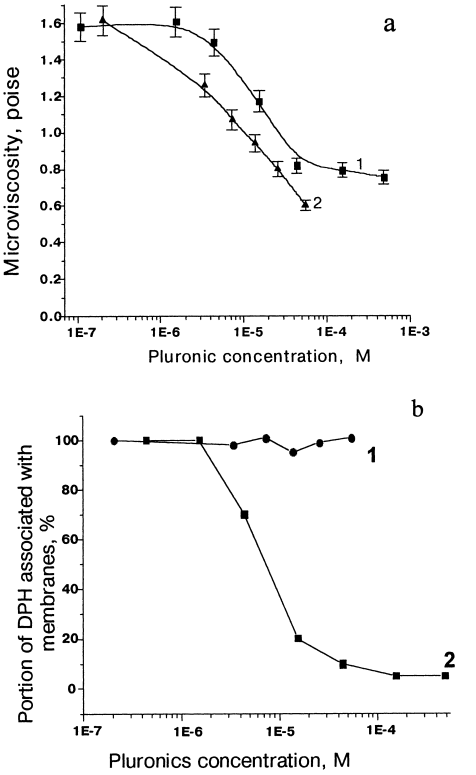

Fig. 3. Influence of L61 (curve 1) and P85 (curve 2) copolymers on the microviscosity of mouse liver microsomes at 37°C (a) and on the portion of DPH associated with membranes (b).

where r_o is the fluorescence anisotropy of the probe at infinitely high viscosity (in polypropylene glycol solution at −70°C), τ is the excited state life-time, T the absolute temperature, $C(r)$ the structural parameter of the probe and η the microviscosity, measured in poise.

Incorporation of DPH into the lipid bilayer resulted in a drastic increase of the fluorescence intensity due to changes of DPH microenvironment polarity. The probe has been previously reported to localize in the hydrophobic region of the biological membrane [25]. Thus, changes in fluorescence anisotropy of DPH give information about structural alterations in the hydrophobic region of the bilayer. Incubation of DPH-labelled mouse liver microsomes with pluronics (Fig. 3a) showed that both P85 and L61 copolymers decreased the membrane microviscosity in a threshold manner. The critical pluronic concentration, which caused an abrupt decrease of microviscosity (3 μM for P85 and 1 μM for L61) corresponds approximately to the critical micellization concentration (CMC) of the pluronic. So it may be supposed that pluronic micelles can extract the probe from the membrane, and therefore, the microviscosity observed at high pluronic concentrations is a characteristic of micelles, not a bilayer. To verify this hypothesis we incubated DPH-labelled microsomes with pluronics, sedimented the microsomes at 46 000 × g (1 h), resuspended the pellet in the initial volume of buffer and measured the fluorescence intensity of the supernatant and the pellet. From these data, the portion of the membrane-bound probe was calculated and presented in Fig. 3b as a function of the pluronic concentration. It could be seen that pluronic P85 extracted the probe from the microsomes, in contrast to pluronic L61 that did not extract DPH. These results indicated

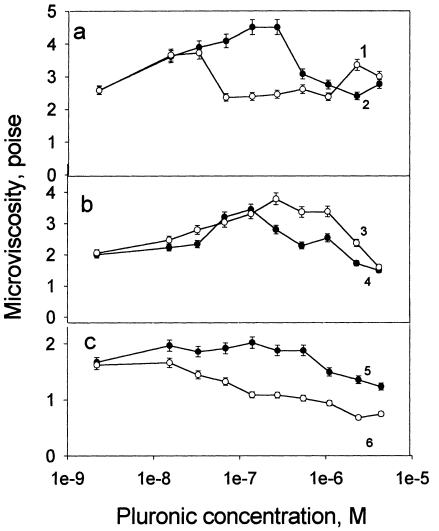

Fig. 4. Influence of pluronics P85 (curves 1, 3, 5) and L61 (curves 2, 4, 6) on the microviscosity of membranes of (a) mouse erythrocytes, (b) splenocytes, (c) myeloma SP2/0 cells. All experiments were performed at 37°C in Hanks' solution.

that the increase of pluronic L61 concentration up to 10 μM did not influence DPH localization in the membrane but induced changes in the fluorescence anisotropy of the probe. Therefore, these changes can be attributed to the alteration of the bilayer structure caused by the pluronic incorporation into the membrane. On the other hand, we implied that pluronic P85 concentration should not exceed 2 μM to avoid DPH redistribution between the membrane and pluronic micelles.

Interaction of both pluronic copolymers with small monolamellar lipid vesicles combined from pure egg yolk phosphatidylcholine and its mixture with cholesterol and phosphatidylserine resulted in small if any changes in membranes microviscosity (results are not shown). So, the membranes built from pure lipids are characterized by low sensitivity to the fluidising effect of pluronic copolymers.

The results given in this paper show that pluronics can bind to cell membranes and induce changes in the packing of membrane lipids. Comparisons of the influence of pluronics on liver microsomes and lipid vesicles show that the effect depends on membrane composition. Therefore, we investigated the pluronic influence on microviscosity of membranes of different blood cells. Fig. 4 shows the pluronics effect on membrane microviscosity of mouse erythrocytes, splenocytes and myeloma SP2/0 cells. Myeloma cell membranes in the absence of pluronics have very low microviscosity that is in good agreement with the previously reported data concerning membrane fluidity of tumor cells [31–33]. The microviscosity of splenocyte and erythrocyte membranes increased in the presence of rather low pluronic concentration (approximately 0.1 μM) and decreased as its concentration reached 1 μM. While the microviscosity of mouse myeloma cells membranes underwent a constant decrease in the 0.1–1 μM pluronic concentration range. No redistribution of DPH between cells and pluronic solution was detected in the studied concentration range. This fact allows us to conclude that pluronic binding to tumor cells membranes resulted in the fluidizing of the bilayer, whereas normal cells microviscosity increases at a therapeutically active pluronic concentration (0.4–1 μM).

4. Discussion

The ability of pluronic copolymers to affect the permeability of biological barriers has been widely discussed in literature [1,2,9–11]. It has been reported that pluronics can influence the biodistribution of anti-tumor drugs enhancing the specificity of their action [16]. In the present work, we evaluated the binding of two pluronic copolymers with normal and tumor blood cells. Binding of both copolymers with all investigated cell types is non-specific and its absolute value is rather low. Only 0.01–0.05% of the added copolymer absorbs on cells in all cases. Bound pluronic molecules occupy no more than 1–3% of cell surface. The binding efficiency depends upon copolymer HLB: binding of the hydrophobic L61 copolymer is 1.5–3 times higher than that of less hydrophobic P85 pluronic. Binding efficiency depends strongly on the nature of cells, pluronics binding with erythrocytes being nearly 10-fold less than with lymphoid cells. It is reasonable to suppose that this effect is mainly due to the difference in the plasma membrane composition of the investigated cells. Erythrocyte plasma membranes are known to contain more cholesterol than the membranes of lymphocytes and tumor cells [34], so it is reasonable to suggest that the fluidity of the membrane determines to some extent its ability to bind pluronic copolymers.

Assuming that binding at 4°C is chiefly caused by adsorption of the polymer on the cell surface, the ability of cells to accumulate pluronic by endocytic uptake at the physiological temperature can be estimated as a difference between the copolymers binding at 37°C and 4°C (Fig. 2c). Investigation of the temperature dependence of the copolymer uptake by different cells revealed peculiarities of the tumor cells. Temperature-dependent uptake of L61 copolymer by SP2/0 myeloma cells at 37°C was twice more effective than at 4°C. It can be supposed that this uptake is due to fluid-phase endocytosis or copolymer diffusion across the membrane. In contrast, normal splenocytes absorb only 20–30% more pluronics at 37°C than at 4°C, indicating that tumor cells can accumulate three times more of the copolymer than normal cells: so binding efficiency of pluronic copolymers with tumor and normal cells is similar, however the character of their association is quite different. Normal cells adsorb most polymers on their surface, whereas tumor cells accumulate them inside.

The observed peculiarities of the copolymers binding with myeloma cells can be mainly determined by the composition and physical properties of the plasma membrane. Therefore, we investigated the influence of the copolymers on plasma membrane microviscosity in different cells. The effect of pluronic copolymers on normal and tumor cell membranes was proved to be quite different. Microviscosity of tumor cells membranes decreased, whereas that of normal cells increased after incubation with rather low (4×10^{-7}–10^{-6} M) pluronic L61 concentrations. P85 copolymer induced similar changes in the membrane microviscosity though less pronounced.

The physico-chemical mechanisms of cell sensitivity to the disturbing action of the macromolecular fluidizer is the subject of further investigations. The preferred fluidizing of tumor cell plasma membranes can be a general basis of selective delivery of drugs in tumor cells.

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References

[1] Lowe, K.C. and Armstrong, F.H. (1990) Adv. Exp. Med. Biol. 277, 267–276.
[2] Omyanagi, H., Saitoh, Y., Uchida, T., Watanabe, M., Yamanouchi, K., Yokoyama, K. and Mitsuno, T. (1992) Biomater. Artific. Cells Immobiliz. Biotech. 20, 941–949.
[3] Forman, M.B., Ingram, D.A. and Murray, J.J. (1992) Am. Heart J. 124, 1347–1357.
[4] Allison, A.C. and Byars, N.E. (1991) Mol. Immunol. 28, 279–284.
[5] Johnston, T.P. and Miller, S.C. (1985) J. Parenter. Sci. Technol. 39, 83–88.
[6] Howell, J.M., Stair, T.O., Howell, A.W., Mundt, D.I., Falcone, A. and Peters, S.R. (1993) Am. J. Emerg. Med. 11, 134–138.
[7] Fults, K.A. and Johnston, T.P. (1990) J. Parenter. Sci. Technol. 44, 58–65.
[8] Johnston, T.P., Dunjabi, M.A. and Froelich, C.J. (1992) Pharmacol. Res. 9, 425–434.
[9] Kabanov, A.V., Slepnev, V.I., Kuznetsova, L.E., Alakhov, V.Y., Batrakova, E.V., Melik-Nubarov, N.S., Sveshnikov, P.G. and Kabanov, V.A. (1992) Biochem. Int. 26, 1035–1042.
[10] Slepnev, V.I., Kuznetsova, L.E., Gubin, A.N., Alakhov, V.Y., Batrakova, E.V. and Kabanov, A.V. (1992) Biochem. Int. 26, 587–595.
[11] Kabanov, A.V., Chekhonin, V.P., Alakhov, V.Y., Batrakova, E.V., Lebedev, A.S., Melik-Nubarov, N.S., Levashov, A.V., Morozov, G.V. and Severin, E.S. (1989) FEBS Lett. 258, 343–345.
[12] Mohamed, F.A.S.T.P. (1995) Pharm. Sci. 5, 456–460.
[13] Misra, A.N. and Rao, V.U. (1996) Indian J. Exp. Biol. 34, 171–173.
[14] Alakhov, V.Y., Moskaleva, E.Y., Batrakova, E.V. and Kabanov, A.V. (1996) Bioconjugate Chem. 7, 209–216.
[15] Venne, A., Li, S., Mandeville, R., Kabanov, A.V. and Alakhov, V.Y. (1996) Cancer Res. 56, 3626–3629.
[16] Batrakova, E.V., Dorodnych, T.Y., Klinskii, E.Y., Kliushnenkova, E.N., Shemchukova, O.V., Goncharova, O.Y., Arjakov, S.A., Alakhov, V.Y. and Kabanov, A.V. (1996) Br. J. Cancer 74, 1545–1552.
[17] MacDonald, R.I. (1985) Biochemistry 24, 4058–4065.
[18] Ohno, H., Shinudzu, N., Tsuchida, E., Sasakawa, S. and Honda, K. (1981) Biochim. Biophys. Acta 649, 221–228.
[19] Yegutkin, G.G. (1997) Membr. Cell Biol. 10, 515–520.
[20] Topchieva, I.N., Osipova, S.V., Banatzkaya, M.I. and Valkova, L.A. (1989) Dokl. Acad. Nauk SSSR (Russ.) 304, 901–913.
[21] Dorfling, P. and Wischner, S. (1984) in: Immunologische Arbeitsmethoden (Friemel, H., Ed.), p. 374, Gustav Fisher Verlag, Jena.
[22] Goldanskii, V.I., Kashirin, I.A., Shishkov, A.V., Baratova, L.A. and Grebenshchikov, N.I. (1988) J. Mol. Biol. 201, 567–574.
[23] Sims, G.E.C. and Snape, T.J. (1980) Anal. Biochem. 107, 60–63.
[24] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[25] Shinitzky, M. and Barenholtz, Y. (1974) J. Biol. Chem. 249, 2652–2657.
[26] Plášek, J. and Jarolim, P. (1987) Gen. Physiol. Biophys. 6, 425–437.
[27] Roozemond, R.C. and Urly, D.C. (1979) Biochim. Biophys. Acta 5566, 17–37.
[28] Evans W.H. (1990) in: Biological Membranes, A Practical Approach (Findley, J.B.C. and Evans, W.H., Eds.), pp. 24–25, IRL Press, Oxford.
[29] Yaroslavov, A.A., Efimova, A.A., Kulkov, V.E. and Kabanov, V.A. (1994) Polymer Sci. (Russian Ed). 36, 264–270.
[30] Mamdouh, Z., Giocondi, M.C., Laprade, R. and Le Grimellec, C. (1996) Biochim. Biophys. Acta 1282, 171–173.
[31] Shinitzky, M. and Inbar, M. (1974) J. Mol. Biol. 85, 603–615.
[32] van Blitterswijk, W.J., Emmelot, E., Hilkmann, H.A.M. and Inbar, M. (1977) Biochim. Biophys. Acta 467, 309–320.
[33] van Hoeven, R.P., van Blitterswijk, W.J. and Emmelot, P. (1979) Biochim. Biophys. Acta 551, 44–54.
[34] Shinitsky, M. and Inbar, M. (1976) Biochim. Biophys. Acta 433, 133–149.