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Enhancement of colloid uptake by tumor cell surface electrical charge modification

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Colloidal [^{51}Cr]chromic phosphate uptake is considerably increased by preincubation of P388 ascites leukemia cells with poly(DL-lysine). The uptake increase is in direct relationship with the concentration and the degree of polymerization of poly(DL-lysine). The probable implication of cell surface electrical charge modification in these phenomena is discussed.

In the process of endocytosis the particle attachment to the cell membrane is of fundamental importance. Signals are relayed to the interior of the cell as attachment takes place and movement of the cytoplasm membrane to form pseudopodes ensues. Thus the extended membrane and cytoplasm tends to fold around the foreign invader, engulfing the material [1]. On the other hand, during radiotherapy using radioactive colloids the attachment of the colloidal particle to the tumor cell surface highly improves the treatment efficiency [2].

The aim of the presen: experimental work has been to assess the effects of polylysine, with different degrees of polymerization, on the in vitro uptake of a radioactive colloid by tumor cells.

Colloidal [^{51}Cr]chromic phosphate was prepared by redox reaction of a mixture of sodium di[^{51}Cr]chromate plus orthophosphoric acid, plus sodium sulfite in the presence of a protective colloid (gelatine) [3]. Laser diffraction determination (Malvern, U.K.) gave a particle size of 55 nm. Poly(DL-lysine)hydrobromide: (a) of relative molecular mass (M_r) 8000, (b) M_r 19000, (c) M_r 27000, (d) M_r 40500 and (e) M_r 77000 were from Sigma Chemical Co., St. Louis, MO, U.S.A.

P388 ascites leukemia cells maintained in the ascitic form by weekly inoculation into B6D2F1 mice were used for these experiments. A week after inoculation, pools of cells from at least five mice were withdrawn for each experiment. After being washed free of ascitic fluid with saline, the cells were suspended in Tyrode medium to give the wanted concentration. A series of five tubes containing 1 ml of cells suspension as preincubate at 37°C for 15 min with the type and concentrations of poly(DL-lysine) to be assayed (0, 25, 50, 100 and 200 μg/ml), centrifuged and the cells washed twice with cold Tyrode. After being resuspended in 1 ml Tyrode, the colloid solution was added, and incubated at 37°C for 30 min. After incubation, the cells were centrifuged, washed twice with cold Tyrode and counted for radioactivity.

The degree of polymerization of poly(DL-lysine), over M_r 8000, affect in a positive way the uptake of colloid (Fig. 1). For all the assayed degrees of polymerization, with the exception of M_r 8000, their increased concentration in the preincubation medium provoked an

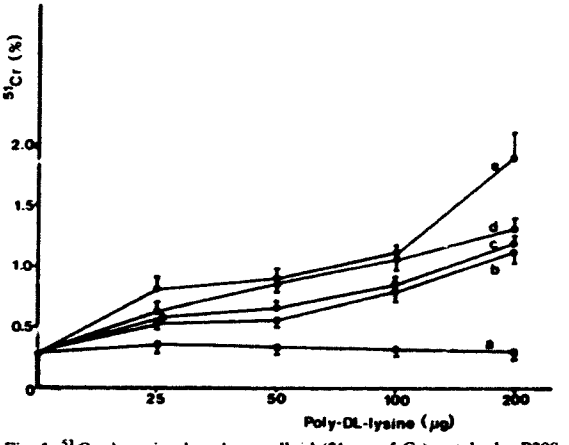

Fig. 1. ^{51}Cr-chromic phosphate colloid (21 μg of Cr) uptake by P388 ascites leukemia cells ($3.5 \cdot 10^7$ cells) preincubated with poly(DL-lysine): (a) M_r 8000, (b) M_r 19000, (c) M_r 27000, (d) M_r 40500 and (e) M_r 77000 (mean value ± S.D. of five incubation tubes).

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increased colloid uptake, specially significant with concentrations higher than 50 μg/ml. The concentration effect is more important for poly(DL-lysine) with higher degrees of polymerization. For example, poly(DL-lysine) M_r 77000 at 100 μg/ml increases the uptake to 3.5-times the corresponding to control cells while at 200 μg/ml it is augmented to six times that value (Fig. 1).

That the colloidal chromic phosphate has a negative electrical charge is inferred from its precipitation when it is mixed with poly(DL-lysine): colloid precipitation is the result of electrical charge neutralization. For this reason the experimental protocol includes a preincubation of tumor cells with poly(DL-lysine) before being in contact with the colloid.

In an earlier report Simon-Reuss et al. [4] pointed out that it was not possible to generalize in regard to the relationship of electrokinetic charge to type of cell, site of origin, rate of growth, or 'malignancy' of the parent tissue. This was concluded from their observation that malignant epithelial HeLa cell is not significantly different from the human epithelial amnion, and from Vassar's [5] demonstration that normal and malignant epithelial cells do not have significantly different surface charge densities. More recently, Price et al. [6] have reported that the cell surface charge becomes more negative on neoplastic transformation, observation that is in agreement with the results of Ambrose et al. [7,8] obtained for a variety of non-transformed and homologous tumor cells. Sialic acid and RNA have been reported to be the principal contributors to the net surface charge [4,6].

To determine the nature and to establish the role of the cell membrane-bound molecular species involved in our colloid uptake was not the aim of this investigation. However, we can consider it very likely that a neutralization and increase in positive electrical charge of the cell membrane by interaction with poly(DL-lysine) is responsible for the colloid uptake augmentation. In our experimental results the effects of concentration and degree of polymerization on colloidal uptake seem in perfect agreement with the concept of an increased positive electrical charge, and that this charge is in direct relationship with concentration and molecular size.

Considering the chemical bases of our colloid preparation, we assume that a carboxyl group from gelatine penetrates the hexaaquo chromic ion. Consequently, it is always possible that other molecules having anion groups capable of replacing water molecules in the inner coordination sphere of Cr(III) can be incorporated as polymolecular structures and that these may behave like the colloidal complex used in this study. This possibility could be of great interest because it would make it feasible to pinpoint tumor cells with molecules of therapeutic action.

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