Effect of Dinitrosyl Iron Complexes on Platelet Aggregation Induced by HeLa Cervical Carcinoma Cells

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 150, No. 9, pp. 341-343, September, 2010
Original article submitted October 20, 2009

We studied the effect of dinitrosyl iron complexes with glutathione ligands on platelet aggregation with HeLa tumor cells. It was shown that dinitrosyl iron complexes not only inhibited cell aggregation, but being added at early stages can also block this process. These findings dictate further studies of dinitrosyl iron complexes as a compound reducing metastasizing and thrombus-formation in tumor patients.

Key Words: *platelets; HeLa; aggregation; dinitrosyl iron complexes with glutathione ligands; nitric oxide*

Activation of platelets under the action of tumor cells and formation of platelet–tumor cell heteroaggregates are, among other sectors, the causes of tumor metastasizing. These heteroaggregates are easily transferred with blood and adhere to endothelium due to the presence of platelets. Moreover, growth factors released by activated platelets can also affect tumor cells. Inhibition of these processes is required for preventing thrombogenic complications and dissemination of the tumor process via metastasizing. Many compounds with antiplatelet activity exhibit also antimetastatic properties. The best studied of them are dipyridamole, prostaglandins, indomethacin, antagonists of β_3-integrins, *etc.* [5]. There are data that NO donors are also effective inhibitors of both platelet aggregation and aggregate formation between platelets and tumor cells [7,8]. Among known NO donors, dinitrosyl iron complexes (DIC) with glutathione ligands attracted much recent attention. Animal experiments demonstrated efficacy of DIC as hypotensive, cardioprotective, and antiplatelet preparation [1,2,4]. Here we present new results on inhibitory action of DIC on the formation of platelet-tumor cell heteroaggregates.

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MATERIALS AND METHODS

DIC were synthesized by the method proposed by A. F. Vanin [1].

Donor blood was obtained from Republican Blood Transfusion Station (Minsk). Platelet-rich plasma was obtained by blood centrifugation at 200*g* at room temperature for 10 min. Platelets were washed by centrifugation of platelet-rich plasma at 1200*g* for 3 min. Platelet suspension was obtained by double washing of the cell pellet with tris-buffer (13.3 mM tris, 120 mM NaCl, 15.4 mM KCl, 6 mM D-glucose, 1.5 mM EDTA, pH 6.9) and stored at room temperature in a concentration of 2.5×10^9 cell/ml.

Tumor cells (HeLa, human cervical carcinoma) were obtained from Institute of Epidemiology and Microbiology (Minsk). Cell culture was grown as described elsewhere [3]. Tumor cells were washed from the culture medium with phosphate buffered saline (10 mM, pH 7.35) and adjusted to a concentration of 10^6 cell/ml at 2-8°C.

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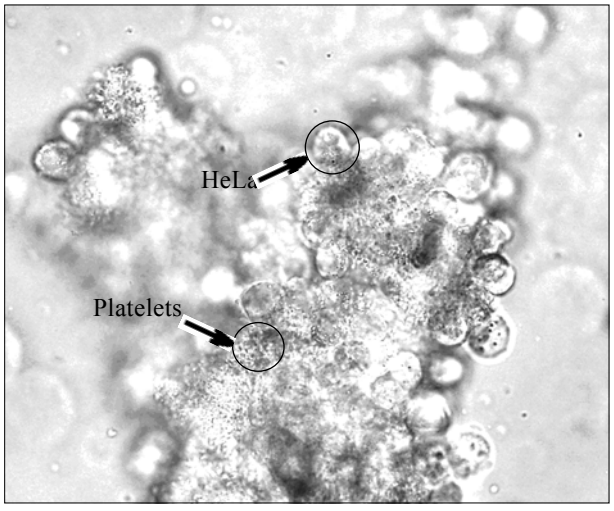

Fig. 1. Platelet-tumor-cell heteroaggregate, ×400.

Platelet aggregation induced by HeLa was studied by optical method using AR2110 analyzer (SOLAR). Washed platelets (30 µl) were added to aggregometer measuring cell containing phosphate buffered saline with Ca^{2+} and Mg^{2+} (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4/KH_2PO_4, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, pH 7.35), incubated at 37°C, agitated for 2 min, and then 10 µl HeLa cell suspension was added. For studying platelet aggregation, changes in light transmission were continuously recorded and the aggregation rate (maximum light transmission, %) and lag-phase (time from addition of tumor cell to the start of aggregation process) were determined.

The formed cell aggregates were studied under an Olympus BX51WI light microscope (×400).

RESULTS

Addition of HeLa cells to platelet suspension was followed by the formation of platelet–tumor cell heteroaggregates (Fig. 1). Typical kinetic curves for platelet aggregation induced by tumor cells are presented (Fig. 2). It should be noted that cell aggregation starts not immediately, but after a certain time interval (15-20 min, lag-phase). Analysis of the effect of DIC on platelet aggregation with tumor cells showed that DIC added to the platelet suspension 2 min before tumor cells dose-dependently inhibited HeLa-induced platelet aggregation, which was seen from longer lag-phase (Fig. 2, *a*). In contrast to lag-phase, the rate and degree of cell aggregation practically did not depend on DIC concentration. Since adhesion receptors such as surface glycoproteins GPIb, GPIIb/IIIa exposed on both platelets and tumor cells can participate in the formation of platelet–tumor cell heteroaggregates [6,10], in a special experimental series platelets or tumor cells were treated with DIC for 2 min and then thoroughly washed from the incubation medium. Inhibition of platelet aggregation with tumor cells was observed

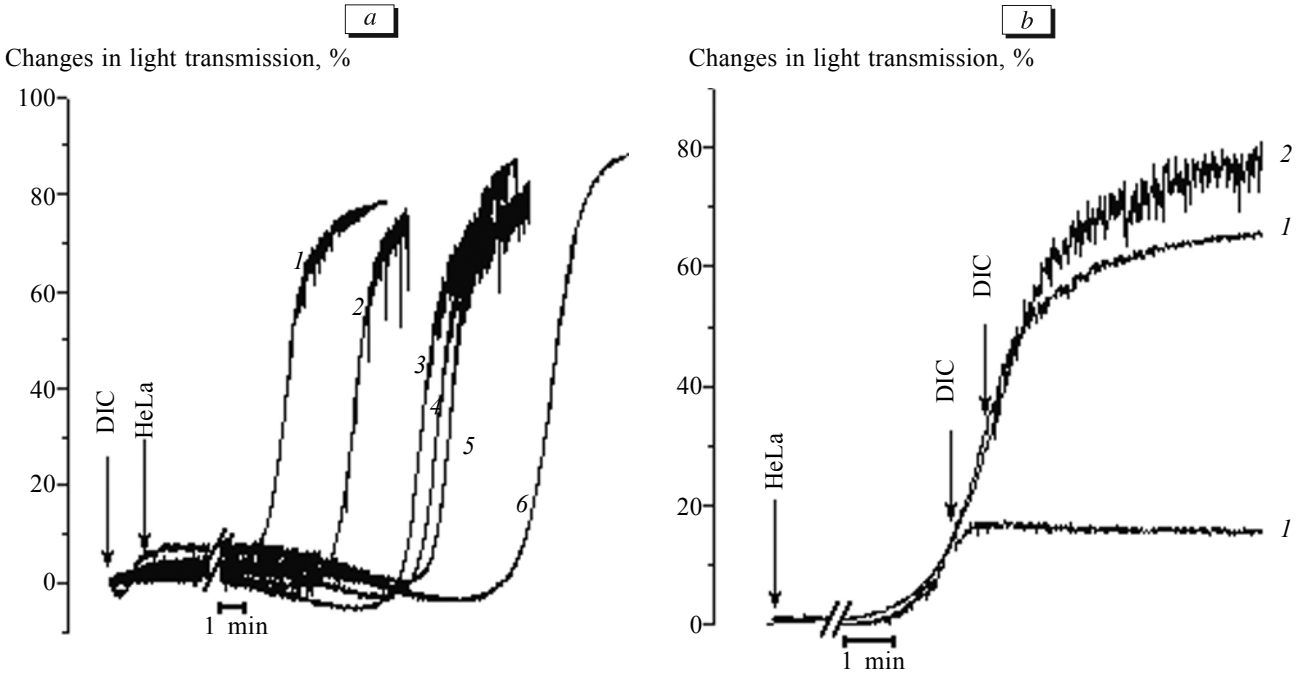

Fig. 2. Effect of DIC on HeLa cell-induced platelet aggregation. *a*) DIC were added to platelet suspension 2 min before addition of tumor cells. *1*) in the absence of DIC; *2*) 0.12 µM; *3*) 0.3 µM; *4*) 0.6 µM; *5*) 1.2 µM; *6*) 2.4 µM DIC. *b*) DIC (60 µM) were added 2 min (first arrow) and 3 min (second arrow) after induction of cell aggregation. *1*) in the presence of DIC; *2*) in the absence of DIC. Typical kinetic curves from 5 independent experiments are presented.

after incubation of either platelets, or tumor cells with DIC. This probably suggests that the inhibiting effect of DIC can be determined by modulation of adhesion properties of both these cell types.

Importantly, tumor cell-induced platelet aggregation was stopped by adding DIC at the early stage of cell aggregation (2 and 3 min after aggregation induction), when light transmission of the cell suspension attained 20-30% (Fig. 2, *b*). Being added at later stages of aggregation, DIC had no effect on the formation of heteroaggregates of the studied cells.

Sulfhydryl groups of adhesion receptors GPIb and GPIIb/IIIa can be potential targets for DIC; nitrosylation of these groups in the reaction with DIC can lead to receptor inactivation and inhibition of cell aggregation. This assumption agrees with the data on inhibiting effect of S-nitrosoglutathione on activation of GPIIb/IIIa receptors [8]. It should be also noted that the process of glycoprotein activation on the cell surface involves matrix metalloproteinase 2 [9] and that NO donors inhibit secretion of this enzyme [7]. Therefore, inhibition of platelets with tumor cells in the presence of DIC can be explained by both their direct effect on adhesion receptors (nitrosylation of sulfhydryl groups) and with inhibition of secretion of matrix metalloproteinase 2 essential for activation of glycoproteins.

Thus, our findings suggest that glutathione DIC are effective inhibitors of tumor cell-induced platelet aggregation. DIC can also help to reduce the rate of tumor metastasizing. We believe that our findings are a prerequisite for further study of DIC as a promising antimetastatic preparation.

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