Effect of the Topoisomerase II Inhibitor Vepeside on the Binding of Doxorubicin with DNA of Anthracycline-Sensitive Tumor Cells

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The effect of the topoisomerase II inhibitor vepeside (VP-16) on the binding of doxorubicin to the DNA of P388 lympholeukemia and Ehrlich's ascitic cancer cells is examined. Direct evaluation of the doxorubicin—DNA binding *in situ* showed that preincubation of the cells with VP-16 stimulates the binding in a dose- and incubation time-dependent manner. Vepeside also increased the binding after it has reached the plateau level; however, the effect was 2-fold lower than that observed after preincubation. Since DNA binding is a factor determining the antitumor activity of anthracyclines, administration of VP-16 should precede that of doxorubicin.

Key Words: *vepeside; doxorubicin; DNA binding; drug interaction*

Drug interactions are a factor which often determines the effectiveness of combined chemotherapy. The significance of this factor increases when drugs with the same cell targets are used. For example, vepeside (VP-16) is often administered in combination with doxorubicin (DR), an inhibitor of DNA-topoisomerase II [4] that controls the DNA structure [9]. On the other hand, interaction with DNA is one of the factors determining the activity of DR [10]. These interactions depend on the DNA structure and, consequently, on the activity of topoisomerase II, which was confirmed by the observation that the activity of this enzyme in resistant tumor cells is changed [2,12]. At the same time, binding of anthracyclines to DNA isolated from resistant cells is lower compared with binding to DNA from sensitive cells [6].

From these data it can be hypothesized that administration of the topoisomerase II inhibitor VP-16 in combination with DR alters DR binding to the DNA of tumor cells, thus changing the antitumor activity of the anthracycline.

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To test this hypothesis we examined the effect of VP-16 on the binding of DR to the DNA of anthracycline-sensitive cells. In an attempt to elucidate the possible mechanisms of VP-16 action we have compared the effects of this inhibitor before DR—DNA binding (preincubation with VP-16 before addition of DR) and after it (addition of VP-16 after the stationary phase of DR—DNA binding had been reached).

MATERIALS AND METHODS

Cells of anthracycline-sensitive murine ascitic tumors (myeloid leukemia P388 and Ehrlich's cancer) and ascitic fluid were used. The cells were maintained by daily intraperitoneal inoculation into mice. The cells were washed two times and suspended in Hanks' solution to a final concentration of 1.5×10^6 cells/ml as estimated by counting in the Goryaev's chamber.

The DR—DNA binding was assessed spectrofluorimetrically. The method is based on fluorescence quenching during incubation with living cells [1,13]. Doxorubicin was added to cell suspension to a final concentration of 2 μM, and its fluorescence was

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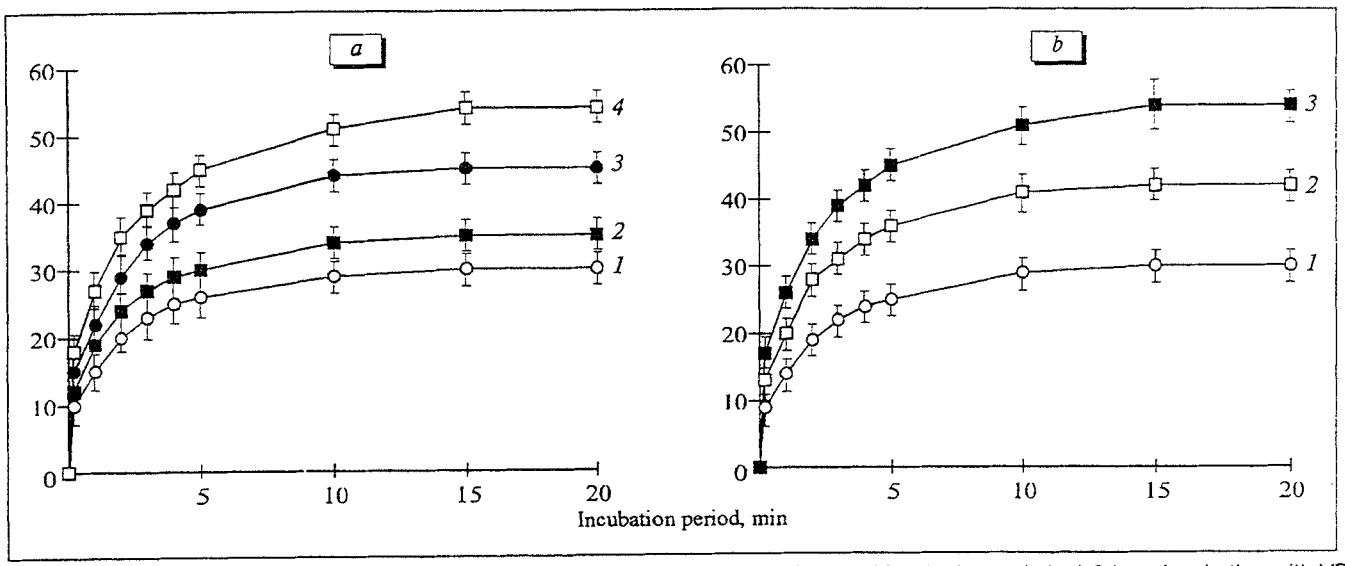

Fig. 1. Binding of doxorubicin (DR) to DNA of P388 cells as a function of VP-16 dose and incubation period. *a*) 2-h preincubation with VP-16; control (*1*); VP-16: 50 (*2*), 100 (*3*), and 200 μg/ml (*4*). *b*) preincubation with 200 μg/ml VP-16; control (*1*), 1 h (*2*) and 2 h (*3*). Here and in Figs. 2 and 3: ordinate: quenching of the DR fluorescence (% of the initial fluorescence).

measured for 20 min at excitation and emission wavelengths of 470 and 590 nm, respectively, in a Hitachi F2000 spectrofluorimeter at an optical slit width 10 nm. Scattered light was cut out with a yellow ZhS-18 filter. Measurements were carried out in a quartz cuvette with an optical pathway length of 1 cm and coefficient of DR absorption <1% per hour. Two protocols were employed. Protocol 1 (preincubation with VP-16): cells were incubated with VP-16 or the same volume of Hanks' solution for 1 or 2 h at 37°C, after which fluorescence was measured. Protocol 2 (postincubation with VP-16): the fluorescence of cell suspensions was measured for 20 min, the cells were then incubated with VP-16 or Hanks' solution, and residual fluorescence was measured.

The results were analyzed by the method of Student—Fischer.

Doxorubicin was from Farmitalia, vepeside was from Bristol-Myers Squibb, and Hanks' solution was from the Institute of Poliomyelitis and Viral Encephalites, Russian Academy of Medical Sciences.

Each experiment was performed in triplicate, each group included at least 3 samples. Results of typical experiments are presented.

RESULTS

Figure 1 illustrates the dependence of DR—DNA on VP-16 dose and preincubation period for P388 cells. Preincubation with VP-16 stimulated the binding, the effect being more pronounced with higher VP-16 doses (Fig. 1, *a*) and longer preincubation periods (Fig. 1, *b*). The maximum quenching of DR fluorescence was observed after a 2-h preincubation with 200 μg/ml VP-16 (maximum plasma concentration attained after a single administration of VP-16 in the therapeutic dose [14]). Therefore, the effects of higher VP-16 concentrations were not studied. Preincubation period was not prolonged due a decrease in cell viability. In further experiments cells were preincubated with 200 μg/ml VP-16 for 2 h.

The effect of preincubation of P388 and Ehrlich's cancer cells with VP-16 on the kinetics of the DR—DNA binding is shown in Figs. 2, *a* and 3, *a*. Enhanced quenching of DR fluorescence in both cell types indicates that the binding of DR to DNA increased under the action of VP-16. In control P388 and Ehrlich's cancer cells, the DR—DNA binding was, respectively, 30±1.5 and 25±10.% of the initial amount of DR added to the cells, while after preincubation with VP-16 it increased to 55±3.0 and 59±1.0% ($p<0.01$). Thus, after preincubation of leukemia P388 and Ehrlich's cancer cells with VP-16 the DR—DNA binding increased, respectively, by 25 and 34% of the initial amount of DR added to the cells.

Incubation of control cells for 2 h at 37°C after the stationary level of DR—DNA binding had been reached practically did not change this parameter in both cell types (Figs. 2, *b* and 3, *b*). However, a 2-h incubation with VP-16 under the same conditions increased the DR—DNA binding in both cell types by about 15% of the initial amount of the anthracycline added to the cells ($p<0.05$), this increase being 1.5- and 2-fold lower, respectively, for P388 and Ehrlich's cancer cells than that observed after preincubation of these cells with VP-16 (Figs. 2, *a* and 3, *a*).

Thus, spectrofluorimetric method of direct evaluation of the DR—DNA binding [1,13] showed that incubation of lympholeukemia P388 and Ehrlich's cancer cells with the DNA topoisomerase II inhibitor VP-16 stimulates binding of DR to DNA.

The drug vepeside (VP-16) produced by Bristol-Myers Squibb is dissolved in a mixture consisting of polyethylene glycol, ethanol, citric acid, and Tween-80. Tween-80 may potentiate the accumulation of anthracyclines in resistant cells as a result of binding to the P-glycoprotein-dependent system of reverse transport of anthracyclines [3]. In the present study we have demonstrated an increase in the binding of DR to the DNA of cells highly sensitive to DR and other preparations (all cells exhibited cross drug resistance, i.e., had a weak expression of P-glycoproteins). Therefore, it can be suggested that the VP-16-induced increase in the DR—DNA binding is not associated with a possible effect of Tween-80 on the intracellular transport of anthracyclines. Taking into account the fact that the components of VP-16 solvent may alter the structure and permeability of the plasma membrane, the effect of VP-16 may be attributed to increased accumulation of DR as a result of the plasma membrane damage. In this case, pre- and postincubation effects of VP-16 should be similar. However, preincubation with VP-16 had a higher stimulatory effect on the DR—DNA binding than

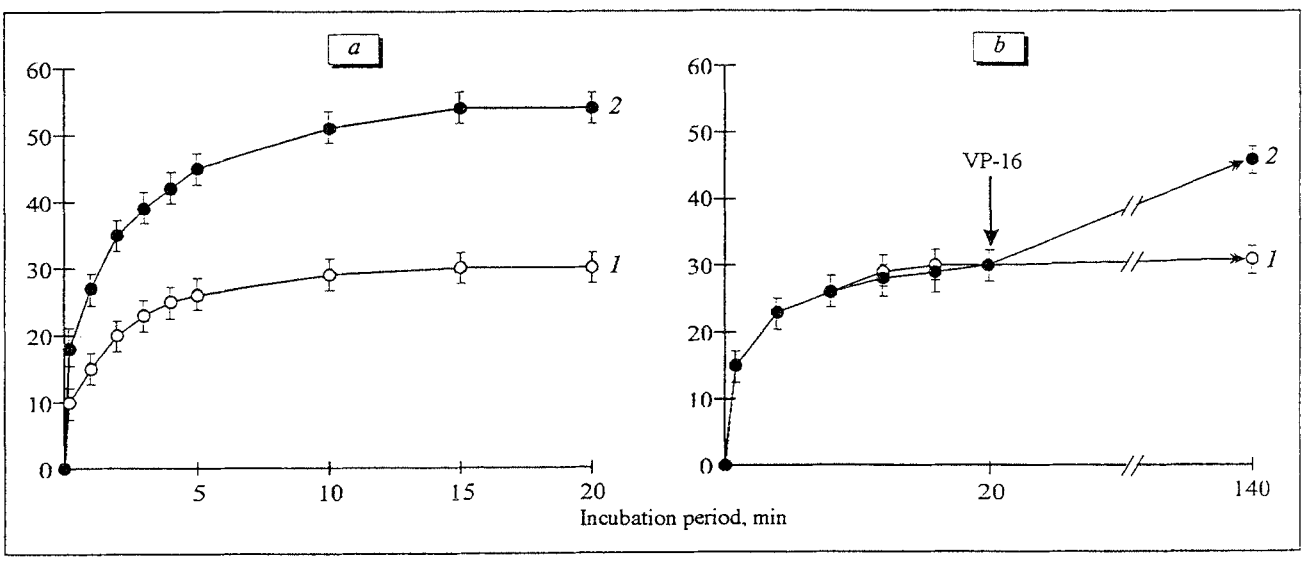

Fig. 2. Effect of preincubation (*a*) and postincubation (*b*) with vepeside (VP-16) on the binding of doxorubicin to DNA of P388 cells. Here and in Fig. 3: *1*) control; *2*) VP-16; 200 μg/ml, 2 h.

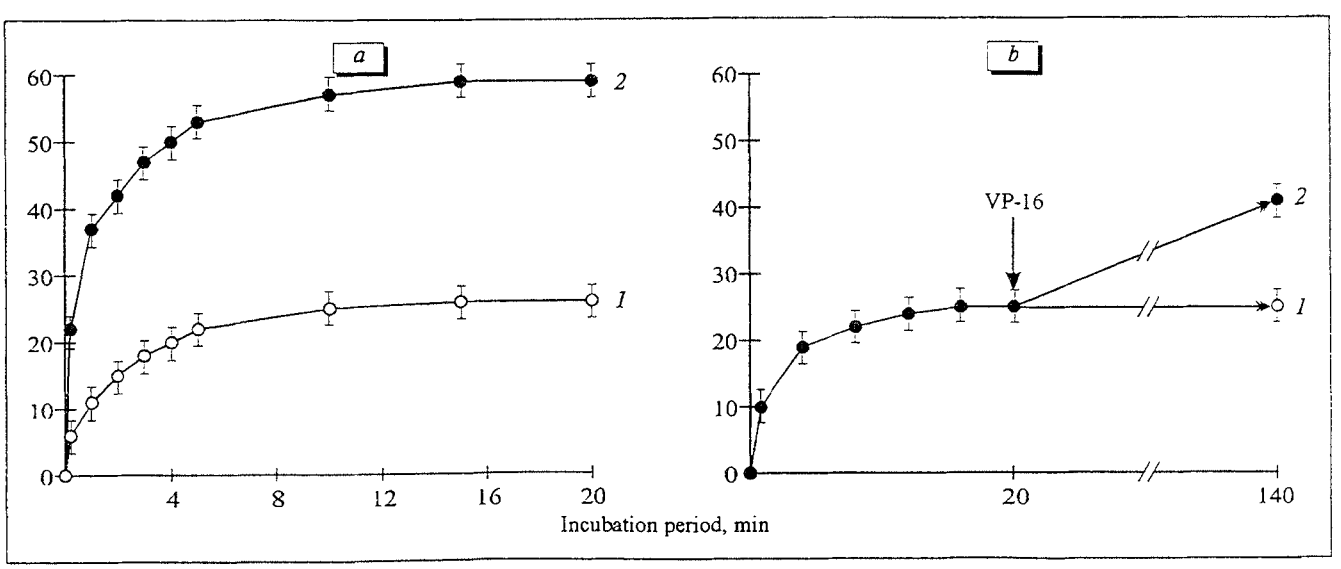

Fig. 3. Effect of preincubation (*a*) and postincubation (*b*) with vepeside (VP-16) on the binding of doxorubicin to the DNA of Ehrlich's cancer cells.

postincubation. Consequently, under the chosen experimental conditions the possible membranotropic effect of the solvent does not increase the DR—DNA binding under the action of VP-16.

It is likely that the modifying effect of VP-16 may result from specific alterations in the DNA structure. The anthracycline VP-16 is a DNA topoisomerase II inhibitor capable of inducing DNA fragmentation in different cell types [7,8,11]. This effect may increase the accessibility of DNA to DR, thus stimulating the DR—DNA binding.

We believe that the lower effect of postincubation with VP-16 on the binding indicates that the ability of VP-16 to induce fragmentation of DNA in the DR—DNA complex decreases, i.e., the DNA becomes less accessible to VP-16. Bearing in mind that fragmentation of intracellular DNA is associated with different specific effects of VP-16 [7,8,11], it can be suggested that in the polychemotherapy schemes in which VP-16 is administered after anthracycline the therapeutic effectiveness of both VP-16 and its combination with the anthracycline is lowered. This suggestion was confirmed by experiments with VP-16 and the anthracycline antibiotic aclarubicin [5].

Thus, the effectiveness of VP-16 combinations with DR and other anthracycline depends on the sequence of drug administration. Since that DNA binding is an important factor determining the antitumor activity of anthracyclines [10], it can be concluded that administration of VP-16 prior to DR increases the effectiveness of DR, while an inverse scheme (VP-16 after DR) may have an opposite effect. Thus, administration of VP-16 prior to DR is preferable.

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