Effect of Verapamil on the Growth and Vincristine Sensitivity of Human Tumors Transplanted under the Mouse Kidney Capsule

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The calcium antagonist verapamil, a P-glycoprotein inhibitor, affects the growth of human tumor transplants under the capsule of the animal kidney with an oscillating-type dose-effect dependence. The maximal positive effect of verapamil is tumor regression and the maximal negative effect stimulation of transplant growth. In 3 out of 6 cases (gastric adenocarcinoma) verapamil at 9 mg/kg injected 0.5 h prior to vincristine inhibited tumor resistance to the latter. These data offer promise for increasing the effectiveness of chemotherapy in patients with cancer of the stomach by means of chemotherapy combined with P-glycoprotein blockers in doses determined for each individual.

Key Words: *human tumors; subcapsular test; verapamil; vincristine*

It is shown that the resistance of tumor cells *in vitro* to such cytostatics as vincaalkaloids and anthracycline antibiotics may be due to their intensified transport from cells effected by P-glycoprotein (P-GP) localized in the plasma membrane [3]. Studies of P-GP-mediated multiple drug resistance performed on continuous cell lines have pinpointed ways of overcoming this problem. Calcium channel blockers [6], calmodulin inhibitors [7], some antiestrogens [8], and an array of other substances have proved to be P-GP inhibitors [5]. While much has been written on the multiple drug resistance phenomenon, its role in human carcinoma chemoresistance remains unclear.

The aim of the present investigation was to study the effect of the P-GP antagonist verapamil (VP) on the growth and vincristine (VC) sensitivity of tumors of the human esophagus, stomach, and lung transplanted under animal kidney capsules.

MATERIALS AND METHODS

Twenty human carcinomas (10 adenocarcinomas of the stomach, 7 nonsmall-cell cancers of the lung, 3 cancers of the esophagus) obtained from operating rooms of the P. A. Gertsen Moscow Research Cancer Institute were studied. Male CBA mice weighing 18-24 g (*Stolbovaya* nursery,

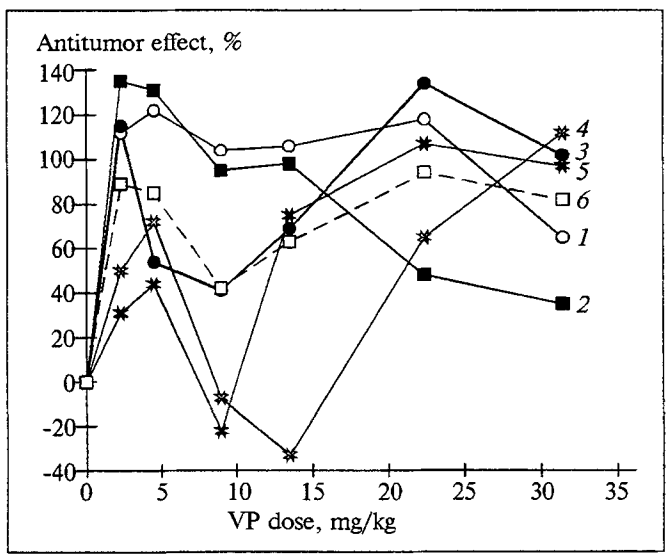

Fig. 1. Effect of VP on the growth of human tumor transplants. 0–100% means inhibition of growth, >100% testifies to regression, and <0 means the stimulation of transplant growth. *1–5*) 5 carcinomas of the stomach; *6*) averaged curve.

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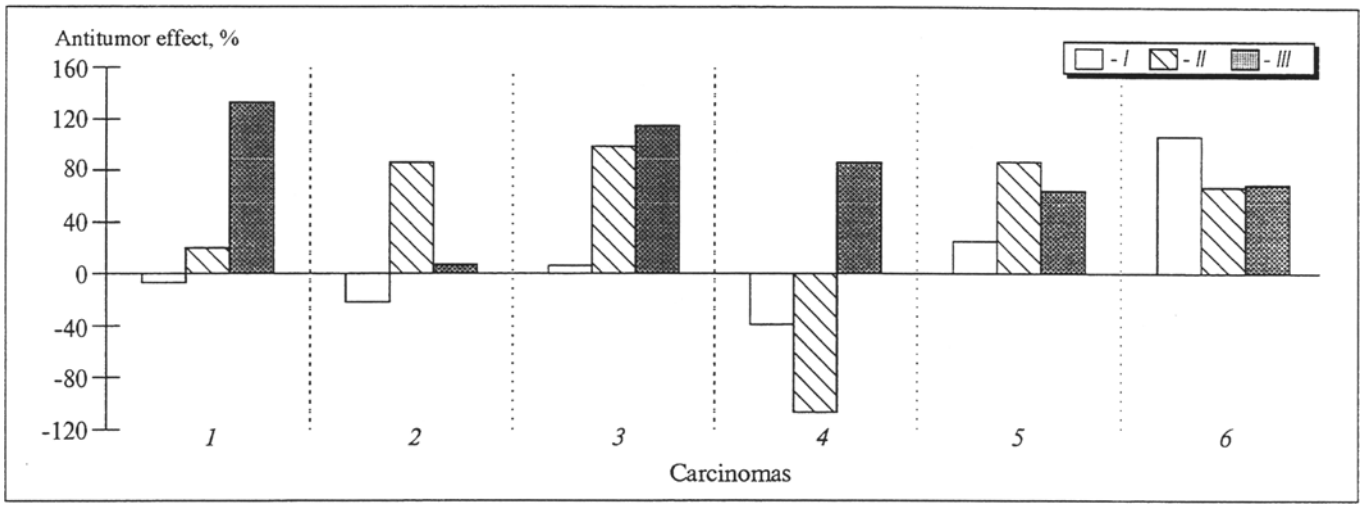

Fig. 2. Effect of VP on the antitumor effect of VC as related to 6 human carcinomas. *I*) effect of VP (9 mg/kg); *II*) effect of VC (2 mg/kg); *III*) actual joint effect of VP and VC.

Russian Academy of Medical Sciences) were used as tumor recipients. The animals underwent a one-trial total gamma-irradiation at 4.5 Gy one day prior to the operation to depress the graft rejection reaction. Fragments of each tumor were implanted under the renal capsule according to a described method [4] in our modification [2]. Each control and test group consisted of 7-9 mice. A total of 700 mice were subjected to the procedure. Animals of the test groups were injected intraperitoneally with VP at 2.3-31.4 mg/kg and VC at 2.0 mg/kg every day starting from the 2nd day after transplantation. At the same times control animals were injected with physiological saline. On the 7th day of the experiment the animals were sacrificed by ether and the mean increase in size of the transplants was assessed in the control and test groups according to an earlier-described method [2]. The effects of the drugs were calculated in percent of the growth inhibition (or regression) of the grafts in the test groups as compared to the controls [1].

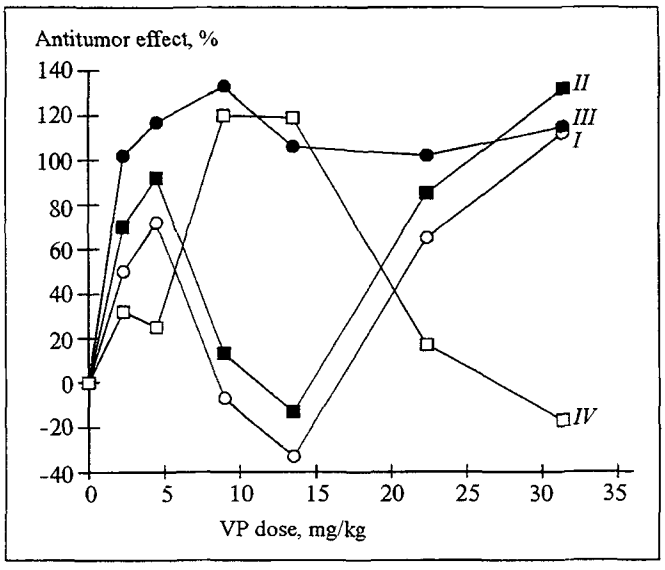

Fig. 3. Effect of VP on VC sensitivity of stomach and lung carcinomas. *I*) effect of VP; *II*) expected joint effect of VP and VC; *III*) actual joint effect of VP and VC; *IV*) difference between expected and actual effects.

RESULTS

On the first stage of the investigation the effect of VP itself on the growth of the 20 tumor transplants was studied. It was shown that VP substantially changes the rate of transplant growth in a dose-dependent manner (Fig. 1). The curves obtained for the different human tumors differed from each other (Fig. 1, *1-5*), although they showed some common features valid for the averaged curve (Fig. 1, *6*). Thus, VP inhibited tumor growth to different degrees at 2.3-4.5 mg/kg. With elevation of the doses, from 4.5 to 13.5 mg/kg, its antiblastic effect diminished (Fig. 1) and in 2 of the 20 cases VP even stimulated tumor growth. In the dose range of 13.5-22.4 mg/kg its antiblastic effect again rose but then, with an increase of the dose to 31.4 mg/kg, it dropped once more. Therefore, the dose-effect dependence for VP has an oscillating character with an individual amplitude of oscillations for each tumor.

The effect of VP on the antiblastic effect of VC was examined in the next stage of the investigation. Six tumors were studied (5 stomach adenocarcinomas and 1 nonsmall-cell lung cancer). Animals were injected VP 0.5 h prior to VC. In 3 of the 6 cases (adenocarcinomas of the stomach) VP abolished tumor VC resistance and the total effect of VP and VC

exceeded the expected one (Fig. 2). One of these cases was analyzed for various VP concentrations (Fig. 3). The dependence of the effect on the VP concentration was oscillating (Fig. 3, *1*). Since the effect of VC proper comprised 20% of the tumor growth inhibition, the expected (for different VP doses) joint effect of VP and VC added together should look like an oscillating curve (Fig. 3, *2*). The actual effect in the range of VP doses of 9.0-13.5 mg/kg was substantially higher than expected (Fig. 3, *3*). The difference between the actual and expected effects of the drugs is shown in Fig. 3, *4*. A superadditivity was evident in the range of VP doses of 9.0-13.5 mg/kg, being maximal at 9.0 mg/kg.

Thus, the calcium antagonist VP, a P-GP inhibitor, is shown to affect the growth of human tumor transplants under the animal renal capsule with a dose-effect dependence of oscillating nature. The maximal positive effect of VC is expressed in tumor regression, the maximal negative effect in the stimulation of transplant growth. In 3 out of 6 cases the use of VP in therapeutic doses resulted in the abolishment of tumor resistance to VC. These findings enable us to hope for an increased effectiveness of chemotherapy in patients with carcinomas of the stomach via an individualized regime of chemotherapy combined with P-GP blockers.

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