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CALCIUM CHANNEL BLOCKERS: POTENTIAL ANTIMETASTATIC AGENTS

KENNETH V. HONN, JAMES M. ONODA,
CLEMENT A. DIGLIO AND BONNIE F. SLOANE

Departments of Radiation Oncology, Radiology, Pathology and Pharmacology, Wayne State University, Detroit, MI 48202

Abstract. The calcium channel blocker nifedipine (Bay A 1040) was examined for its effects on tumor cell-platelet interactions. In vitro, nifedipine inhibited tumor cell-induced platelet aggregation and platelet enhanced tumor cell adhesion to confluent endothelial cell monolayers and in vivo nifedipine inhibited pulmonary tumor colony formation ("experimental metastasis") by intravenously injected tumor cells. This evidence suggests that calcium channel blockers may be a new class of antimetastatic agents.

Both human and animal tumor cells have been reported to induce aggregation of platelets (1,2). The association between platelets and tumor cells may facilitate metastasis. It has been suggested that the resultant tumor cell-platelet thrombus could protect the tumor cells from attack by the host immune system, increase the likelihood that the tumor cells would adhere to the endothelial lining of the vascular system and protect adherent tumor cells from dislodgement (3). Pharmacological agents that inhibit platelet aggregation or reduce platelet number have been shown to suppress spontaneous metastasis and pulmonary tumor colony formation (4). The induction of platelet aggregation is known to require both intracellular and extracellular Ca++ (5,6). This suggests that calcium channel blockers which prevent the influx of extracellular Ca^{++} in several cell types may affect platelet aggregation and, in fact, it has been reported that calcium channel blockers inhibit epinephrine and ADP induced platelet aggregation (7,8). We report here our study of the effects of nifedipine (Bay A 1040), a calcium channel blocker, on the interactions between tumor cells and platelets in vitro and on their presumptive interactions in vivo. Our results suggest that calcium channel blockers inhibit tumor cell-platelet interactions and thus may represent a new class of antimetastatic agents.

Materials and Methods. Tumor Cells. B16 amelanotic melanoma (B16a) and Walker 256 carcinosarcoma (W256) were obtained from the Division of Cancer Treatment, Animal and Human Tumor Bank. Tumors were maintained by s.c. implantation into syngeneic male C57BL/6J mice (B16a) or allogenic female Sprague Dawley rats (W256). Animals were housed under identical conditions of temperature, feeding, photoperiod, etc. Tumors were routinely restarted from liquid N_2 frozen stock after 6 isotransplants. Viable (>90%) tumor cell suspensions were prepared from primary subcutaneous tumors by sequential collagenase digestion and separated from normal host cells (<3% contamination) and non-viable tumor cells by centrifugal elutriation as previously described (9).

Platelet Preparation. Platelet rich plasma (PRP) was obtained from heparinized human blood or citrated Sprague Dawley rat blood as previously described (10). Washed rat platelets and rat platelet poor plasma (PPP) were prepared from PRP as previously described (10).

Cultured Endothelial Cells. A virally transformed line of rat cerebral microvasculature endothelial cells was prepared as previously described by Diglio et al. (11).

Tumor Cell Adhesion Studies. Freshly dispersed W256 tumor cells were adapted for growth in tissue culture

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medium. Cells were labeled with ^{125}I-Udr (0.5 μCi/ml) for 24 hr, harvested with trypsin (0.25% plus 0.02% EDTA), pelleted and resuspended. For adhesion studies 16 mm Costar multiwell plates containing confluent rat endothelial cells were used. Each well had a constant volume containing (where appropriate) 2.5 x 10^4 tumor cells, 3 x 10^8 WRP, PPP (0.1%, v/v), 2 mM $CaCl_2$, nifedipine (40 μg/ml), polyethlene glycol-400 solvent and Ca^{++} and Mg^{++}-free media to equalize volumes. Nifedipine or solvent control was added immediately prior to plating. The tumor cells were allowed to adhere for one hr at 37°C and the experiments were terminated by the removal of non-adhering tumor cells by vacuum aspiration. The wells were washed twice with MEM buffer and trypsin was added to remove adherent tumor cells for counting.

"Experimental Metastasis". In the lung colony assay, C57BL/6J mice were pretreated with nifedipine or solvent control (p.o.) one hr prior to the tail vein injection of 25,000 elutriated B16a tumor cells. Mice were sacrificed 21 days later, the lungs were removed, fixed in Bouin's solution and macroscopically visible tumor colonies were counted.

Nifedipine was generously provided by Pfizer, Brooklyn, NY.

Results. Elutriated B16a cells induced aggregation of human PRP following a short lag period (Figure 1). Nifedipine produced a dose-dependent inhibition of this aggregation.

We examined the possibility that platelet-tumor cell interactions are bidirectional and may result in alterations in the tumor cell which would enhance adherence to the blood vessel wall without resulting in platelet aggregation. The availability of rat endothelial cells allowed us to use a completely homologous system for adhesion studies. The ability of WRP to enhance the adhesion of ^{125}I-Udr labeled W256 cells to endothelial cells was determined under four conditions: A) W256 cells alone, B) W256 cells + WRP, C) W256 cells + 0.1% (v/v) PPP and D) W256 cells + WRP + PPP. We have previously demonstrated that tumor cells can only induce aggregation of WRP in the presence of at least 0.1% PPP (v/v; data not shown).

Platelets significantly increased the adhesion of W256 cells to endothelial cells (Figure 2). Platelets

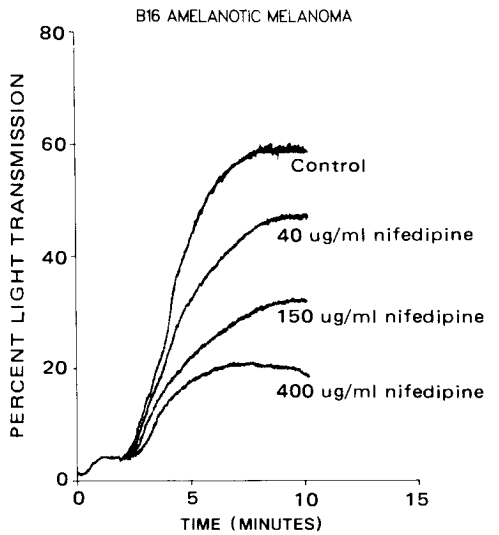

Fig. 1. Inhibition of B16a induced aggregation of human PRP by nifedipine. Nifedipine or solvent control was added to each aggregometry cuvette 1 min prior to the addition of tumor cells.

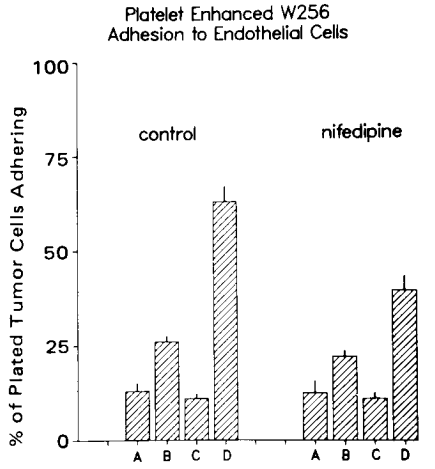

Fig. 2. Inhibition of tumor cell adhesion to confluent cultures of rat endothelium by nifedipine. Each striped bar represents the mean of 6 replicate wells (± SEM) and is representative of triplicate experiments.

enhanced the percentage of W256 cells adherent to endothelium 100% in the absence of PPP (non-aggregatory conditions) and 473% in the presence of PPP (aggregatory conditions), suggesting that platelets might enhance tumor cell adherence to the blood vessel wall even in the absence of overt platelet aggregation (thrombi formation). However, platelet aggregation further enhanced tumor cell adherence to cultured endothelial cells (Figure 2). We tested the effects of nifedipine on platelet enhancement of W256 cell adhesion to endothelial cells. Nifedipine (40 μg/ml) significantly reduced the enhancement by platelets of tumor cell adhesion to endothelial cells under both non- aggregatory (15% inhibition) and aggregatory (38% inhibition) conditions (Figure 2).

We have previously shown that inhibitors of platelet aggregation *in vitro* (i.e., prostacyclin) have potent antimetastatic effects *in vivo* (12). Therefore, we tested nifedipine for antimetastatic properties *in vivo*. For these studies we used a syngeneic murine tumor model (B16a) to study hematogenous metastasis. Although this model system does not represent the complete metastatic cascade (13), it is useful in demonstrating that a drug is affecting transient interactions between intravenously injected tumor cells and host cells rather than having multiple effects on host cells and the primary tumor. A single administration of nifedipine produced a dose-dependent inhibition of lung colony formation (Figure 3). Fifty percent inhibition was observed at 10 mg/kg body weight.

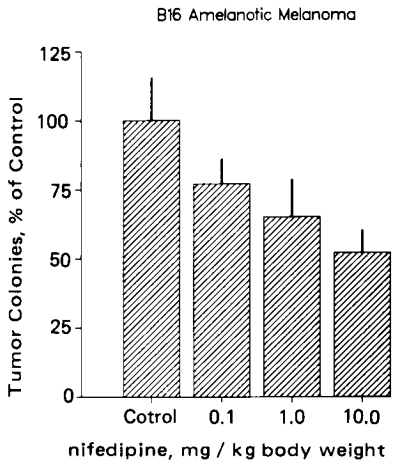

Fig. 3. Inhibition of pulmonary B16a tumor colony formation by nifedipine. The data is presented as the mean ± SEM for each of the groups examined [values in the bars represent the median (range) of metastases for each group]. Each group consisted of 10 mice.

Discussion. Interactions between host platelets and tumor cells have been thought to facilitate metastasis. However, a causal relationship has not been established. The mechanisms that have been suggested for platelet enhancement of metastasis have all assumed that platelet aggregation is required. The ability of platelets to enhance tumor cell adhesion to endothelial cells suggests that platelets may facilitate metastasis in the absence of, as well as, in the presence of overt aggregation. We have previously reported that several agents which inhibit tumor cell-induced platelet aggregation or platelet enhanced tumor cell adhesion to endothelium are antimetastatic *in vivo* (4,12,14). This is the first report that a calcium channel blocker inhibits tumor cell-induced platelet aggregation and platelet enhanced tumor cell adhesion *in vitro* and lung colony formation *in vivo*. We speculate that calcium channel blockers may represent a new class of pharmacological agents which limit tumor cell-platelet interactions and thus can function as antimetastatic agents.

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