Inhibition of tumor cell–platelet interactions and tumor metastasis by the calcium channel blocker, nimodipine

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Nimodipine, a dihydropyridine calcium channel blocker, was evaluated in vitro for its ability to inhibit platelet aggregation induced by B16 amelanotic melanoma (B16a) and Walker 256 carcinosarcoma (W256) cells, and for its ability to inhibit platelet-enhanced B16a and W256 adhesion to rat microvascular endothelial cells. Nimodipine produced a dose-dependent inhibition of tumor-cell-induced platelet aggregation (TCIPA). Platelets enhanced tumor cell adhesion to endothelium both in the presence and absence of overt platelet aggregation. However, the greatest enhancement of adhesion occurred under aggregatory conditions. Nimodipine at a dose of 40 µg/ml inhibited platelet-enhanced adhesion to endothelium under aggregatory and nonaggregatory conditions. Nimodipine was tested in vivo for its ability to inhibit both 'experimental' and spontaneous metastasis. Nimodipine produced a 46 per cent inhibition of lung colony formation at a dose of 5 mg/kg body-weight. Over a dose range of 0.1–80 mg/kg, nimodipine produced a significant dose-dependent inhibition in the formation of lung metastases from a subcutaneous tumor. The in vitro results demonstrate that a dihydropyridine calcium channel blocker can inhibit tumor cell–platelet–endothelial cell interactions. The in vivo results suggest that these compounds may be a new class of antimetastatic agent.

Introduction

Interactions between tumor cells and platelets are thought to facilitate metastasis by enhancing tumor cell arrest in the microvasculature or tumor cell adhesion to the blood-vessel wall [24]. Although the mechanisms by which platelets might enhance metastasis are not yet fully delineated, several laboratories have demonstrated that both human and animal tumor cells can induce platelet aggregation in vitro [1, 12, 16, 20, 29, 45] and that i.v. injection of tumor cells results in thrombocytopenia in vivo [10, 13]. Gasic et al. [10, 11] reported that in the presence of thrombocytopenia (induced by neuraminidase or antiplatelet antiserum) the number of lung colonies produced upon tail-vein injection of TA3 ascites tumor cells was reduced. In this 'experimental metastasis' model the concomitant infusion of platelets prevented the thrombocytopenia-induced decrease in number of lung colonies [11]. We have shown that prostacyclin (PGI2), the most potent platelet antiaggregatory agent known, is also a potent inhibitor of platelet aggregation induced by rodent tumor cells in vitro [18, 23] and of lung colony formation in vivo [17, 19]. Lerner et al. [30]

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have demonstrated that PGI$_2$ can also inhibit platelet aggregation induced by several human tumor cell lines. Recently we have suggested that overt platelet aggregation may not be required in order for platelet–tumor cell interactions to enhance metastasis [20]. In a homologous system we found that rat platelets enhanced the adhesion of W256 cells to plastic substratum both in the absence and presence of overt platelet aggregation [20]. Prostacyclin inhibited this platelet-enhanced tumor cell adhesion [20].

Platelet aggregation is known to require both intracellular and extracellular Ca$^{2+}$ [4, 14, 38, 39]. It would seem intuitively obvious that calcium channel blockers which prevent the influx of extracellular Ca$^{2+}$ in several cell types [3, 25] should inhibit platelet aggregation. Several calcium channel blockers have been demonstrated to inhibit platelet aggregation induced by a variety of stimuli [37, 40, 41]. In this study, we demonstrated that a dihydropyridine calcium channel blocker can inhibit TCIPA and platelet-enhanced tumor cell adhesion in vitro in addition to inhibiting metastasis in vivo.

**Materials and methods**

**Tumor lines**

B16 amelanotic melanoma (B16a) and Walker 256 carcinosarcoma (W256) tumors were obtained from the Division of Cancer Treatment (National Cancer Institute) tumor bank. Tumors were passaged in vivo by s.c. injection of cellular brei into syngeneic male hosts (C57BL/6J; Jackson Laboratory, Bar Harbor, ME) for B16a and into allogenic female hosts (Sprague–Dawley rats; Spartan Laboratory, Lansing, MI) for W256. In order to maintain their metastatic phenotype, all tumor lines were routinely restarted from liquid N$_2$ frozen stocks after six isotransplant generations.

**Tumor cell preparation**

Cell suspensions were prepared from s.c. tumors by sequential collagenase digestion and tumor cells were purified by centrifugal elutriation as previously described [22, 43]. Tumor cells were counted with a model ZBI Coulter counter and viabilities determined by vital dye exclusion. Final monodispersed tumor cell suspensions were > 95 per cent viable and contained < 3 per cent contaminant host cells and no cellular debris.

**Platelet preparation**

Human platelet-rich plasma (PRP) was prepared from a freshly drawn vena puncture sample from the antecubital vein of aspirin-free donors. Blood was drawn using an 18 gauge needle at a heparin to blood ratio of 1 : 9 (v/v). The heparin solution consisted of 50 units/ml heparin in 4-8 per cent (w/v) dextrose buffered by 25 mM Hepes, pH 7.5. Heparinized rat PRP was prepared in similar fashion or blood was drawn from the inferior vena cava into 3-8 per cent (w/v) sodium citrate (1 : 9; v/v) for preparation of washed rat platelets (WRP) and rat platelet poor plasma (PPP) according to the method of Mustard et al. [36]. Washed platelets were recalcified (2 mM Ca$^{2+}$) prior to use in adhesion studies.

**Platelet aggregometry studies**

Aggregometry studies were performed with a Sienco DP247E dual channel aggregometer. Final tumor cell concentrations were held constant at $5 \times 10^5$/cuvette.