Biochemical Pharmacology, Vol. 34, No. 2, pp. 235–241, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

INHIBITION BY DIHYDROPYRIDINE CLASS CALCIUM CHANNEL BLOCKERS OF TUMOR CELL–PLATELET–ENDOTHELIAL CELL INTERACTIONS *IN VITRO* AND METASTASIS *IN VIVO*

KENNETH V. HONN,*†‡ JAMES M. ONODA,* KIM PAMPALONA,* MARJORIE BATTAGLIA,* GREGORY NEAGOS,* JOHN D. TAYLOR,*† CLEMENT A. DIGLIO§ and BONNIE F. SLOANE*‖

Departments of *Radiation Oncology, †Biological Sciences, §Pathology and ‖Pharmacology, Wayne State University, Detroit, MI 48202, U.S.A.

(*Received* 23 *January* 1984; *accepted* 30 *May* 1984)

Abstract—Three calcium channel blockers of the dihydropyridine class were tested *in vitro* for their effects on tumor cell–platelet–endothelial cell interactions and *in vivo* for antimetastatic properties. Felodipine, nimodipine and nifedipine inhibited tumor cell-induced platelet aggregation *in vitro* in a dose-dependent manner. These compounds also inhibited platelet-enhanced tumor cell adhesion to endothelial cells *in vitro*. Lung colony formation ("experimental" metastasis) and spontaneous pulmonary metastasis were inhibited by felodipine, nimodipine and nifedipine. From the present studies on three calcium channel blockers of the dihydropyridine class we hypothesize that calcium channel blockers may represent a new generic class of antimetastatic agents.

A distinguishing feature of malignant tumors is their ability to metastasize to distant sites via the circulatory or lymphatic systems. In man, the presence of tumor cells in the circulation does not necessarily indicate a poor prognosis [1], suggesting that a critical event in the hematogenous phase of the metastatic cascade may be tumor cell arrest and formation of stable adhesions to the endothelium or de-endothelianized surfaces. In our laboratories we have been exploring the possibility that interactions among tumor cells, platelets and endothelial cells facilitate tumor cell arrest and adhesion [2, 3]. In an animal model, Gasic and co-workers [4, 5] have shown that induction of thrombocytopenia via neuraminidase or antiplatelet antiserum reduces lung colony formation by intravenously injected tumor cells, whereas concomitant platelet infusion prevents this decrease in lung colony formation. We have demonstrated that a number of agents which inhibit platelet aggregation [exogenous and endogenous prostacyclin (PGI_2), prostacyclin stimulating agents (nafazatrom) and thromboxane (TX) synthase inhibitors] are antimetastatic in animal tumor models [2, 3, 6, 7].

Both intracellular and extracellular Ca^{2+} seem to be required for irreversible platelet aggregation [8, 9]. Thromboxane A_2 is a platelet proaggregatory agent which may act in part by releasing intracellular stores of Ca^{2+} in the platelet [10, 11], whereas PGI_2, a platelet antiaggregatory agent, acts in part by increasing intracellular sequestration of Ca^{2+} in the platelet [8]. Calcium channel blockers, which prevent the influx of extracellular Ca^{2+} in several cell types [12–14], have been shown recently to inhibit ADP-, epinephrine- and collagen-induced platelet aggregation *in vitro* [15–17], perhaps due to inhibition of intracellular Ca^{2+} mobilization [17]. In humans with coronary heart disease, administration of nifedipine, a dihydropyridine class calcium channel blocker, has been shown to decrease ADP- and collagen-induced aggregation of platelets *ex vivo* and to increase bleeding time [18]. The ability of calcium channel blockers to inhibit platelet aggregation *in vitro* and *ex vivo*, in conjunction with our previous studies indicating that antiplatelet agents are antimetastatic, led us to evaluate three calcium channel blockers of the dihydropyridine class for their abilities to inhibit tumor cell–platelet–endothelial cell interactions *in vitro* and 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 subcutaneous 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. All tumor lines were routinely restarted from liquid N_2 frozen stocks after six isotransplant generations in order to maintain their metastatic phenotype.

Tumor cell preparation. Cell suspensions were prepared from subcutaneous tumors by sequential collagenase digestions [19], and tumor cells were purified by centrifugal elutriation as previously described [20]. Tumor cells were counted with a

‡ Address all correspondence to: Dr. Kenneth V. Honn, Division of Cancer Biology, Wayne State University School of Medicine, 210 Science Hall, 5045 Cass St., Detroit, MI 48202.

model ZBI Coulter counter and viabilities were determined by Trypan blue dye exclusion. Final monodispersed tumor cell suspensions were >90% viable and contained <3% contaminant host cells and no cellular debris.

Platelet preparation. Platelet rich plasma (PRP) was obtained from heparinized human blood or citrated Sprague–Dawley rat blood as previously described [21]. Washed rat platelets (WRP) and rat platelet poor plasma (PPP) were prepared from PRP as previously described [21].

Cultured endothelial cells. A Rous sarcoma virus transformed endothelial cell line was derived from primary rat cerebral endothelial cells as described by Diglio *et al.* [22].

Platelet aggregometry studies. Aggregometry studies were performed with a Sienco DP247E dual channel aggregometer. Final tumor cell concentrations were 5×10^5/cuvette and final platelet concentrations 7.5×10^7/cuvette. All experiments were run in triplicate as individually controlled samples.

Tumor cell adhesion studies. Freshly dispersed B16a and W256 tumor cells were adapted for growth in tissue culture medium as previously described [23] and subcultured one time for use in these experiments. Labeling was accomplished by exposing cells to [^{125}I]deoxyuridine (0.5 μCi/ml) for 24 hr. Cells were harvested with 0.25% (w/v) trypsin plus 0.02% (w/v) EDTA, quenched with 10% (v/v) fetal calf serum, washed twice, and resuspended in tissue culture medium. Viabilities as determined by Trypan blue dye exclusion were >97%. An aliquot of the final tumor cell suspension was counted in a Searle 1185 dual channel gamma scintillation spectrometer. The average specific activity of labeling was 300,000 cpm/10^6 tumor cells.

For adhesion studies, 16 mm Costar multiwell plates or 16 mm Costar multiwell plates containing confluent monolayers of transformed rat endothelial cells were used. Each well had a constant volume containing (where appropriate) 2.5×10^4 tumor cells, 3×10^8 WRP, PPP (0.1%, v/v), 2 mM $CaCl_2$, felodipine (100 μM), nifedipine (100 μM), nimodipine (100 μM), polyethylene glycol-400 solvent and Ca^{2+}- and Mg^{2+}-free media to equalize volumes. Drug or solvent control was added immediately prior to plating. The tumor cells were allowed to adhere for 30 min at 37° and the experiments were terminated by the removal of non-adherent tumor cells by vacuum aspiration. The adherent cells were washed, harvested as described above, and counted for radioactivity. The number of adherent tumor cells was calculated from the specific activity of radiolabeling and expressed as a percentage of the total cells added to each well. Media blanks were less than 0.1% of the total counts. Each condition (A through D) was run in six replicates, and each experiment was repeated from two to three times with comparable results.

Metastasis studies. Tumor cells were purified by centrifugal elutriation as described above for use in both "experimental" and spontaneous metastasis studies. In the "experimental" metastasis studies (lung colony formation), animals were pretreated (p.o. by intubation) with felodipine, nimodipine, nifedipine or solvent control (0.1 ml) 1 hr prior to and 1 hr post tail vein injection of 3.5×10^4 B16a cells. No further drug was administered. Animals were killed 21 days post tumor cell injection, lungs were removed and fixed in Bouin's solution, and macroscopic colonies were counted as previously described [3, 6]. In the spontaneous metastasis studies, animals were injected subcutaneously with 1×10^5 B16a cells into the right axillary region. Drug or solvent control (0.1 ml) treatment (p.o by intubation) was begun 24 hr post tumor cell injection and continued 1× daily for 27 days. Animals were killed on day 28, lungs were removed and fixed, and macroscopic pulmonary metastases were counted as previously described [3, 6]. Twelve animals were used per treatment group, and experiments were repeated from two to four times with comparable results.

Chemicals. Nimodipine was provided by Dr. Alexander Scriabine, Miles Institute for Preclinical Pharmacology (West Haven, CT, U.S.A.), nifedipine by Pfizer (Brooklyn, NY, U.S.A.) and felodipine by Hassle (Molndal, Sweden).

Statistical analysis. *In vitro* assays were analyzed using analysis of variance. Significantly different ($P \leq 0.05$) groups were confirmed using Scheffe's test. *In vivo* experimental data were analyzed using the Kruskall-Wallis test. Significantly different ($P \leq 0.05$, chi distribution) groups were confirmed using the Q test [24].

RESULTS

Inhibition of tumor cell-induced platelet aggregation. Irreversible aggregation of human PRP was induced *in vitro* by elutriated murine B16a cells (Figs. 1 and 2) or by elutriated rat W256 cells (Fig. 1). Three calcium channel blockers of the dihydropyridine class (felodipine, nimodipine and nifedipine) inhibited, in a dose-dependent manner, the induction of platelet aggregation by W256 cells (Fig. 1) and by B16a cells (Figs. 1 and 2).

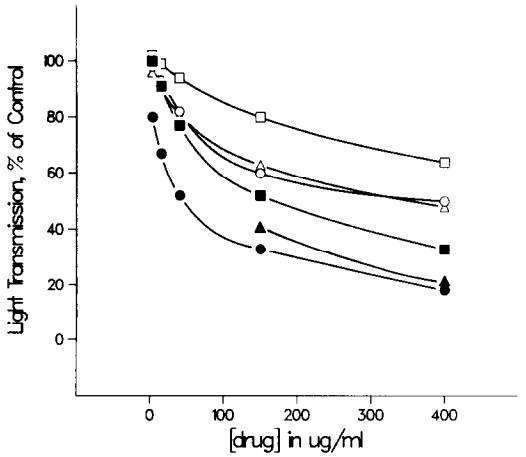

Fig. 1. Dose-dependent inhibition by felodipine, nimodipine and nifedipine of aggregation of heparinized human PRP induced by B16a and W256 tumor cells. Solid symbols, B16a; open symbols, W256; (○, ●) nimodipine; (△, ▲) felodipine; and (□, ■) nifedipine.

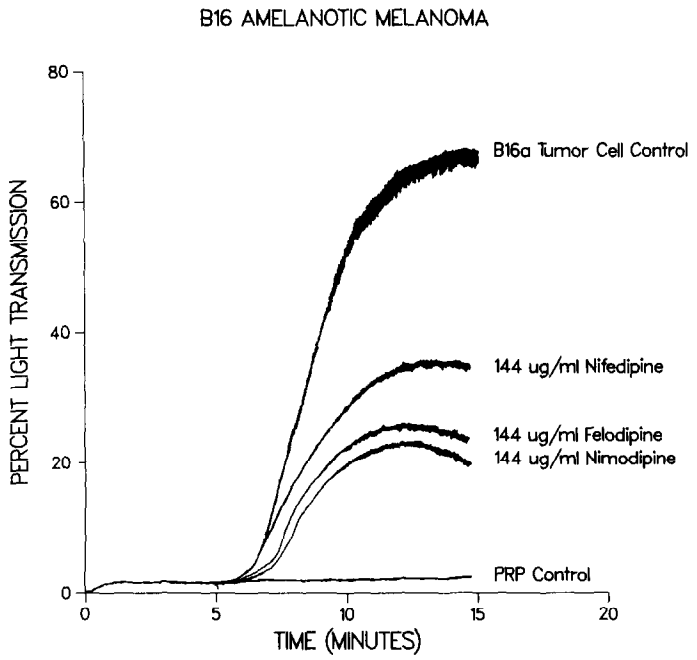

Fig. 2. Representative tracings demonstrating inhibition of B16a-induced aggregation of heparinized human PRP by felodipine, nimodipine and nifedipine.

Nimodipine and felodipine were approximately equipotent as inhibitors of tumor cell-induced platelet aggregation. The IC_{50} for inhibition by nimodipine of B16a-induced platelet aggregation was 85 μM and of W256-induced platelet aggregation was 900 μM. For inhibition by nifedipine the IC_{50} values were 400 μM (B16a) and 1450 μM, (W256), and for inhibition by felodipine the IC_{50} was 880 μM (W256). Nimodipine was also inhibitory in a homologous system consisting of rat tumor cells (W256) and rat PRP prepared from heparinized blood; the IC_{50} was 25 μM. The degree of inhibition by the calcium channel blockers was inversely related to the strength of the aggregating stimulus (tumor cell concentration) and varied with platelet donor.

For the experiments described above, the dihydropyridine class calcium channel blockers were incubated with PRP for 2 min prior to the addition of tumor cells. Lengthening the preincubation period to 10–15 min did not affect the inhibition or the relative inhibitory potency of the two drugs (data not shown). In addition, the inhibition of tumor cell-induced platelet aggregation was not reversible for up to 20 min following the addition of tumor cells (data not shown).

Inhibition of platelet-enhanced tumor cell adhesion to endothelial cells. We have reported previously that rat platelets (washed) enhance the adhesion of rat W256 cells to monolayers of normal endothelial cells (derived from rat cerebral microvasculature) under non-aggregatory and aggregatory conditions [25]. This enhancement of adhesion did not require overt platelet aggregation, yet was potentiated if platelet aggregation was allowed to occur (confirmed by scanning electron microscopy). Platelet-enhanced adhesion of W256 cells to both normal and transformed rat endothelial cells is quantitatively similar [25]. Adhesion of murine B16a cells to transformed rat endothelial cells is also enhanced by platelets under non-aggregatory and aggregatory conditions [25].

In the present study, we demonstrated that the adhesion of murine B16a cells and rat W256 cells to plastic culture dishes was increased in the presence of platelets (Fig. 3). Adhesion of B16a cells was not increased significantly under non-aggregatory conditions (absence of PPP; Fig. 3, Control B) but was increased 79% under aggregatory conditions (presence of 0.1% PPP; Fig. 3, Control D). Previous studies [26] have demonstrated that a variety of tumor cell lines require a plasma factor (PPP) to induce aggregation of washed platelets. Adhesion of W256 cells was increased 85% under aggregatory conditions (Fig. 3, Control D). Adhesion of tumor cells to endothelial cells was also tested using a homologous system of rat W256 tumor cells, rat platelets (WRP), and monolayers of transformed endothelial cells derived from rat cerebral microvasculature. Adhesion of W256 cells to endothelial cells was increased in the presence of platelets (Fig. 4). Tumor cell adhesion was increased 100% under non-aggregatory conditions (Fig. 4, Control B) and 385% under aggregatory conditions (Fig. 4, Control D).

The three dihydropyridine class calcium channel blockers were tested for their abilities to inhibit platelet-enhanced W256 cell adhesion to endothelium, whereas nimodipine was tested for its ability to inhibit platelet-enhanced B16a and W256 cell adhesion to plastic. Platelet-enhanced adhesion to plastic of both B16a and W256 cells was inhibited by nimodipine at a dose of 100 μM (Fig. 3, Nimo-

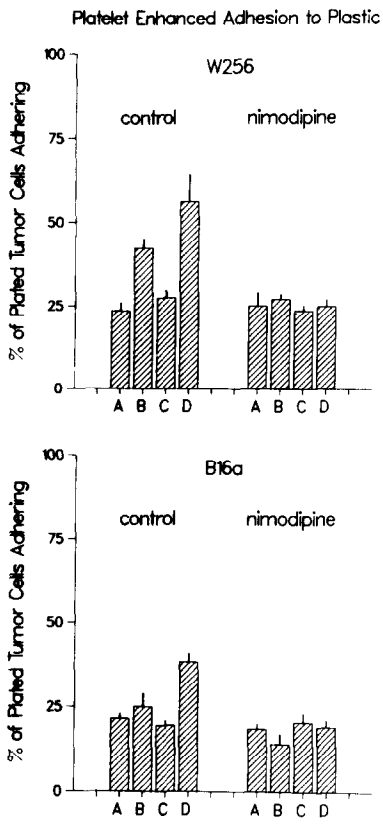

Fig. 3. Representative experiment demonstrating inhibition by nimodipine (100 μM) of rat platelet-enhanced adhesion of B16a melanoma and W256 carcinosarcoma cells to plastic. Key: (A) tumor cells alone, (B) tumor cells + platelets, (C) tumor cells + 0.1% PPP, and (D) tumor cells + platelets + PPP. Values are expressed as $\bar{x} \pm$ S.E.M.; N = 6.

dipine B and D); basal adhesion to plastic was not inhibited (Fig. 3, Nimodipine A and C). Similar results were obtained with felodipine and nifedipine (data not shown). Nimodipine also inhibited platelet-enhanced W256 cell adhesion to endothelial cells (Fig. 4, Nimodipine B and D), but not basal adhesion (Fig. 4, Nimodipine A and C). Thus, the inhibitory effect of nimodipine on tumor cell adhesion does not seem to be limited to either artificial or natural substratum. The two other dihydropyridine class calcium channel blockers also inhibited W256 cell adhesion to endothelial cells. Felodipine at a dose of 100 μM inhibited both platelet-enhanced W256 cell adhesion to endothelial cells and basal adhesion of W256 cells to endothelial cells (Fig. 4). Felodipine and nimodipine were more potent inhibitors than was nifedipine, resulting in a >90% inhibition of platelet-enhanced W256 cell adhesion to endothelium under non-aggregatory (B) and aggregatory (D) conditions. Nifedipine at a dose of 100 μM did not inhibit significantly platelet-enhanced W256 cell adhesion to endothelium under non-aggregatory (B) conditions but did inhibit adhesion by 38% under aggregatory (D) conditions.

Metastasis. Since we have demonstrated previously that compounds which inhibit interactions among tumor cells, platelets and endothelial cells *in vitro* have antimetastatic properties when administered *in vivo* [3, 6, 7], we tested the dihydropyridine class calcium channel blockers for antimetastatic activity *in vivo* using spontaneous and "experimental" metastasis models. The "experimental" metastasis model, using intravenously injected tumor cells, enabled us to examine the effects of felodipine, nimodipine and nifedipine on the interactions among circulating tumor cells, platelets and endothelial cells. Results obtained in the "experimental" metastasis model were then confirmed by daily administration of felodipine, nimodipine or nifedipine in a spontaneous model (metastasis from a subcutaneous tumor) in which the drugs may exert multiple effects, i.e. on the primary tumor as well as on circulating tumor cells or on host cells (platelets). In the studies of both "experimental" and spontaneous metastasis, we used only the syngeneic murine B16a tumor model and not the allogenic rat W256 tumor model.

A single administration of nimodipine (10 mg/kg body wt, p.o. by intubation) 1 hr prior to and 1 hr post intravenous injection of elutriated B16a tumor

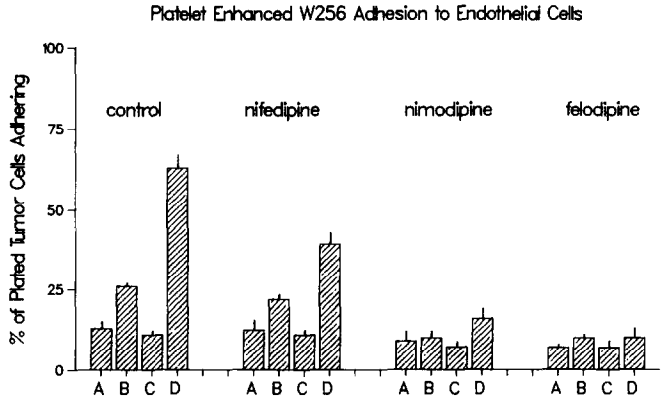

Fig. 4. Representative experiment demonstrating inhibition by felodipine, nimodipine and nifedipine (100 μM) of rat platelet-enhanced adhesion of W256 carcinosarcoma cells to a virally transformed line of endothelial cells derived from rat cerebral microvasculature. Key: (A) tumor cells alone, (B) tumor cells + platelets, (C) tumor cells + 0.1% PPP, and (D) tumor cells + platelets + PPP. Values are expressed as $\bar{x} \pm$ S.E.M.; N = 6.

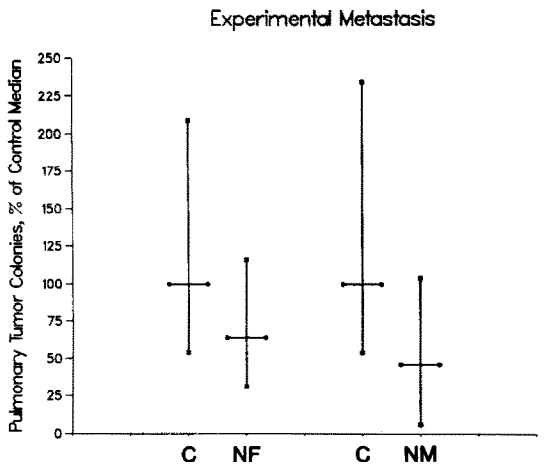

Fig. 5. Inhibition by nifedipine (NF) and nimodipine (NM), at 10 mg/kg body wt, of pulmonary tumor colony formation by intravenously injected B16a cells. Values are expressed as median and range (as a percentage of control) from at least three representative experiments (N = twelve animals per group) and are significantly different from control ($P \leq 0.005$).

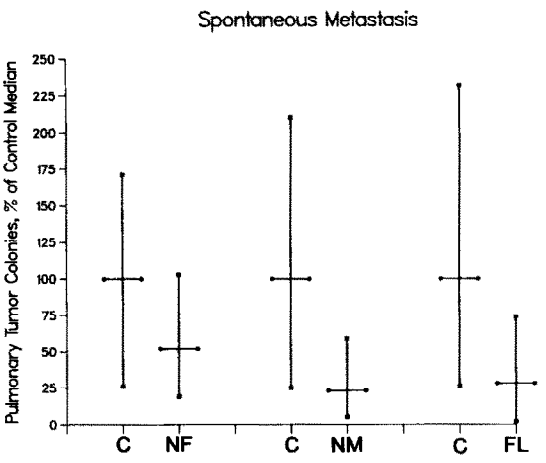

Fig. 7. Inhibition by felodipine (FL), nifedipine (NF) and nimodipine (NM) of pulmonary metastasis from a subcutaneous B16a tumor. Values are expressed as median and range (as a percentage of control) from three representative experiments (N = twelve animals per group) and are significantly different from control ($P \leq 0.005$).

cells resulted in a 57% decrease in lung colony formation (Fig. 5), whereas nifedipine at the same dose decreased lung colony formation by 33% (Fig. 5). In additional studies employing lower drug concentrations (0.1 to 4.0 mg/kg body wt), nimodipine was also a more effective inhibitor of lung colony formation than was nifedipine (Fig. 6). The antimetastatic effects of felodipine were only tested at the lowest drug concentration (0.1 mg/kg body wt). At this dose the ability of felodipine to inhibit lung colony formation (43% decrease) was not significantly different from that of nimodipine (34% decrease; Fig. 6).

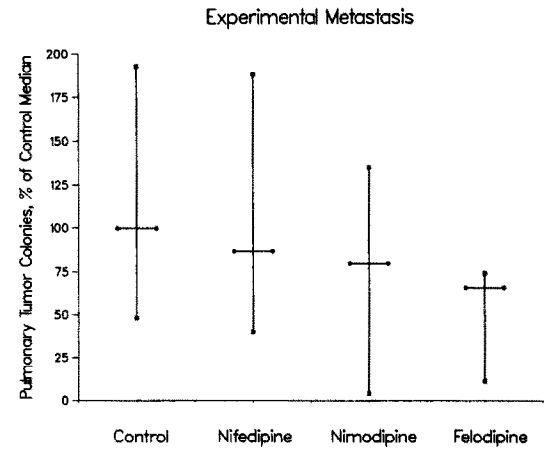

Fig. 6. Inhibition by felodipine, nimodipine and nifedipine, at 0.1 mg/kg body wt, of pulmonary tumor colony formation by intravenously injected B16a cells. Values are expressed as median and range (as a percentage of control) from representative experiments (N = twelve animals per group). Values for felodipine and nimodipine are significantly different from control ($P \leq 0.05$).

Daily administration of drug (10 mg/kg body wt, p.o. by intubation) to mice bearing subcutaneous B16a tumors resulted in a 70% reduction in the number of pulmonary metastases by felodipine, a 72% reduction by nimodipine, and a 40% reduction by nifedipine (Fig. 7). The three calcium channel blockers were not toxic to the mice over a period of 27 days at doses of $\leq$80 mg/kg body wt. No significant differences were observed in the body weight of mice in the present study (Table 1). The effects of the three dihydropyridine class calcium channel blockers on spontaneous metastasis could not be attributed to effects on the primary tumor as neither the wet weights nor the volumes of the primary subcutaneous tumors were reduced significantly (Table 1).

DISCUSSION

Calcium ions serve as molecular messengers which regulate intracellular biochemical functions. Calcium antagonists by preventing release of Ca^{2+} from intracellular stores or influx of external Ca^{2+} can interfere with these biochemical functions. One group of calcium antagonists have as their primary action an effect on the "slow channels" in the cell membrane through which calcium enters the cells [13]. This class of compounds originally described by Fleckenstein [13] was named calcium channel blockers. Currently known calcium channel blockers belong to four chemical classes: (1) dihydropyridine (nimodipine), (2) phenylalkylamines (verapamil); (3) diphenylalkylamines (prenylamine) and (4) benzothiazepines (diltiazem). Due to their inhibition of Ca^{2+} influx and thereby contractility in vascular smooth muscle, these compounds have received widespread use in the treatment of cardiovascular disease in Europe [27, 28] and are now in clinical use (verapamil, diltiazem, nifedipine) or in clinical trials for use in the U.S.A. (nimodipine).

Table 1. Effects of calcium channel blockers on primary tumor weight and volume

	Volume (mm^3)	Weight (g)	Mouse weight
Control	1767 ± 93	1.56 ± 0.1	21.2 ± 0.7
Nimodipine*	1583 ± 156	1.61 ± 0.2	21.7 ± 0.5
Control	2791 ± 673	2.52 ± 0.4	20.5 ± 0.9
Nifedipine*	2262 ± 229	2.08 ± 0.3	19.8 ± 0.5
Control	1420 ± 128	1.3 ± 0.2	21.8 ± 0.6
Felodipine*	1105 ± 139	1.03 ± 0.13	22.4 ± 0.5

* Drug was administered 1× daily (p.o. by intubation for 28 days).

Recently, calcium channel blockers have been found to enhance conventional antitumor therapy. Calcium channel blockers of the phenylalkylamine and benzothiazepine classes have been demonstrated to enhance the cytotoxicity of drug resistant lines derived from several murine solid tumors to standard chemotherapeutic agents such as adriamycin, vincristine and vinblastine [29, 30]. In addition, verapamil has been demonstrated to increase (>50%) blood flow to a murine adenocarcinoma at levels which did not significantly alter arterial blood pressure [31]. These results suggest that calcium channel blockers may provide a means of improving the delivery of chemotherapeutic agents to solid neoplasms and may, in addition, have the potential to increase the effectiveness of radiation therapy by increasing oxygenation of the tumor hypoxic cell fraction.

The experimental results presented in this paper suggest that compounds of the dihydropyridine class of calcium channel blockers are potential antimetastatic agents. We have previously proposed and demonstrated that several antiplatelet agents (e.g. PGI_2, PGI_2-stimulating agents and TX synthase inhibitors) possess antimetastatic properties [6, 32]. Although representatives of each chemical class of calcium channel blockers have been found to inhibit platelet aggregation, the dihydropyridines and phenylalkylamines appear to be the most potent [15, 33]. We have demonstrated that dihydropyridine class calcium channel blockers inhibit tumor cell-induced platelet aggregation and platelet-enhanced tumor cell adhesion to plastic or endothelial cells *in vitro* and tentatively suggest that their antimetastatic effects *in vivo* may be related to inhibition of tumor cell–platelet–endothelial cell interactions. The abilities of felodipine and nimodipine to inhibit tumor cell–platelet–endothelial cell interactions *in vitro* were approximately equipotent as were their abilities to inhibit metastasis *in vivo*. Nifedipine was a substantially less effective inhibitor both *in vitro* and *in vivo*.

We do not, however, exclude the possibility that the dihydropyridines may be antimetastatic *in vivo* due to their effects on hemodynamic parameters [34], to inhibition of phosphodiesterase [35], to inhibition of calmodulin [36] or to stimulation of PGI_2 production by pulmonary endothelial cells [37]. Nevertheless, we believe that the antimetastatic effects of the calcium channel blockers are not due to the hypotensive properties of these compounds. Prostacyclin and prostaglandin E_2 are approximately equipotent as vasodilators; however, only PGI_2 is antimetastatic [6]. In addition, we have tested three other hypotensive agents (minoxidil, prazosin, trimethaphan) as potential antimetastatic agents in the "experimental" metastasis model. None of these agents possessed antimetastatic activity (unpublished observations). Inhibition of phosphodiesterase or of calmodulin and stimulation of PGI_2 would, of course, all result in inhibition of platelet aggregation. We have shown previously that PGI_2 inhibits tumor cell-induced platelet aggregation and platelet-enhanced tumor cell adhesion *in vitro* and lung colony formation *in vivo* [2, 3, 6]. Phosphodiesterase inhibitors have been reported to be antimetastatic *in vivo* although Maniglia *et al.* [38] suggested that the antimetastatic effects of the phosphodiesterase inhibitor 2,6 - bis(diethanolamino) - 4 - piperidinopyrimido[5, 4-*d*]pyrimidine (RA233) are not due to antiplatelet activity nor to inhibition of primary tumor growth. RA233 did not inhibit "experimental" metastasis but did inhibit spontaneous metastasis [38]. In the present study, the dihydropyridines exhibited antimetastatic effects in the absence of effects on primary tumor growth in a spontaneous metastasis model and also exhibited antimetastatic effects in an "experimental" metastasis model.

At low concentrations calcium channel blockers reduce the transmembrane transport of extracellular Ca^{2+}. However, at higher concentrations these agents can also effect cellular mobilization of Ca^{2+} [39]. The effects of the dihydropyridine class calcium channel blockers on inhibition of tumor cell-induced platelet aggregation and of platelet-enhanced tumor cell adhesion to endothelium reported here could be due to inhibition of either platelet transmembrane Ca^{2+} transport or platelet intracellular Ca^{2+} mobilization. We cannot exclude either possibility. The inhibition of ADP- and collagen-induced platelet aggregation by verapamil and nifedipine has been reported to be due to inhibition of intracellular Ca^{2+} mobilization [17]. However, there is an obligatory requirement for extracellular Ca^{2+} for induction of platelet aggregation by tumor cells [26]. The ability of dihydropyridine class calcium channel blockers to inhibit tumor cell-induced platelet aggregation may be due in part to inhibition of intracellular Ca^{2+} mobilization and/or in part to inhibition of platelet transmembrane Ca^{2+} transport.

These studies indicate that three representatives of the dihydropyridine class of calcium channel

blockers have antimetastatic properties. Preliminary results in our laboratories indicate that representatives of two other classes of calcium channel blockers (phenylalkylamines and benzothiazepines) inhibit tumor cell-induced platelet aggregation and platelet-enhanced tumor cell adhesion to endothelium. Although calcium channel blockers represent four chemical classes and at least two mechanisms of action, we propose that calcium channel blockers may represent a new generic class of antimetastatic agents. This proposal is attractive in that calcium channel blockers (1) have been selected for low chronic toxicity due to their intended long-term use in cardiovascular patients, (2) can be administered orally, (3) are currently in clinical trial or have been approved for use in treatment of cardiovascular disease in the U.S.A. and, therefore, could be readily available for Phase I clinical trials in cancer if efficacy in animal models can be demonstrated, (4) may synergize with PGI_2-stimulating agents and thromboxane synthase inhibitors (which lower platelet intracellular Ca^{2+}), compounds which are currently under development as antimetastatic agents, and (5) may, at the same time, enhance conventional cytotoxic chemotherapy and radiation therapy.

Acknowledgements—K. V. H. thanks the Departments of Chemistry and Biological Sciences for providing laboratory space used in these studies. This work was supported by a special grant from Wayne State University, Office of the Provost.

REFERENCES

1. A. J. Salsbury, *Cancer Treat. Rev.* **2**, 55 (1975).
2. K. V. Honn, D. G. Menter, J. M. Onoda, J. D. Taylor and B. F. Sloane, in *Cancer Invasion and Metastasis: Biologic and Therapeutic Aspects* (Eds. G. L. Nicolson and L. Milas), p. 361. Raven Press, New York (1984).
3. K. V. Honn, *Clin. expl Metastasis* **1**, 103 (1983).
4. G. J. Gasic, T. B. Gasic and C. C. Stewart, *Proc. natn. Acad. Sci. U.S.A.* **61**, 46 (1968).
5. G. J. Gasic, T. B. Gasic, N. Galanti, T. Johnson and J. Murphy, *Int. J. Cancer* **11**, 704 (1973).
6. K. V. Honn, B. Cicone and A. Skoff, *Science* **212**, 1270 (1981).
7. K. V. Honn, J. Meyer, G. Neagos, T. Henderson, C. Westley and V. Ratanatharathorn, in *Interaction of Platelets and Tumor Cells* (Ed. G. A. Jamieson), p. 295. Alan R. Liss, New York (1982).
8. N. E. Owen, H. Feinberg and G. C. LeBreton, *Am. J. Physiol.* **239**, H483 (1980).
9. N. E. Owen and G. C. LeBreton, *Am. J. Physiol.* **241**, H613 (1981).
10. O. V. Miller, R. A. Johnson and R. R. Gorman, *Prostaglandins* **13**, 599 (1977).
11. R. R. Gorman, *Fedn Proc.* **38**, 83 (1979).
12. T. Godfraind, *Fedn Proc.* **40**, 2866 (1981).
13. A. Fleckenstein, *Arzneimittel-Forsch.* **20**, 1317 (1970).
14. E. Braunwald, *New Engl. J. Med.* **307**, 1618 (1982).
15. H. Ono and M. Kimura, *Arzneimittel-Forsch.* **31**, 1131 (1981).
16. G. A. Schmunk and A. M. Lefer, *Res. Commun. Chem. Path. Pharmac.* **35**, 179 (1982).
17. P. Han, C. Boatwright and N. G. Ardlie, *Thromb. Haemostas.* **50**, 513 (1983).
18. J. Dale, K. H. Landmark and E. Myhre, *Am. Heart J.* **105**, 103 (1983).
19. K. V. Honn, M. Romine and A. Skoff, *Proc. Soc. exp. Biol. Med.* **166**, 562 (1981).
20. B. F. Sloane, J. R. Dunn and K. V. Honn, *Science* **212**, 1151 (1981).
21. P. G. Cavanaugh, B. F. Sloane, A. Bajkowski, G. J. Gasic, T. B. Gasic and K. V. Honn, *Clin. expl Metastasis* **1**, 297 (1983).
22. C. A. Diglio, D. E. Wolfe and P. Meyers, *J. Cell Biol.* **97**, 15 (1983).
23. B. F. Sloane, K. V. Honn, J. G. Sadler, W. A. Turner, J. J. Kimpson and J. D. Taylor, *Cancer Res.* **42**, 980 (1982).
24. J. H. Zar, *Biostatistical Analysis*, 2nd Ed. Prentice-Hall, Englewood Cliffs, NJ (1984).
25. K. V. Honn, J. M. Onoda, C. A. Diglio, M. M. Carufel, J. D. Taylor and B. F. Sloane, *Clin. expl Metastasis* **2**, 61 (1984).
26. Y. Hara, M. Steiner and M. G. Baldini, *Cancer Res.* **40**, 1217 (1980).
27. P. D. Henry, *Am. J. Cardiol.* **46**, 1047 (1980).
28. R. R. Hope and R. Lazzara, *Adv. intern. Med.* **27**, 435 (1982).
29. T. Tsuruo, H. Iida, K. Naganuma, S. Tsukagoshi and Y. Sakurai, *Cancer Res.* **43**, 808 (1983).
30. T. Tsuruo, H. Iida, S. Tsukagoshi and Y. Sakurai, *Cancer Res.* **42**, 4730 (1982).
31. W. G. Kaelin, S. Shrivastav, D. G. Shand and R. L. Jirtle, *Cancer Res.* **42**, 3944 (1982).
32. K. V. Honn, W. D. Busse and B. F. Sloane, *Biochem. Pharmac.* **32**, 1 (1983).
33. L. G. T. Ribeiro, T. A. Brandon, J. K. Horak, J. A. Ware, R. R. Miller and R. T. Solis, *J. cardiovasc. Pharmac.* **4**, 170 (1982).
34. R. P. Hof, A. Hof and P. Neumann, *J. cardiovasc. Pharmac.* **4**, 352 (1983).
35. J. A. Norman, J. Ansell and M. A. Phillipps, *Eur. J. Pharmac.* **93**, 107 (1983).
36. S. L. Bostrom, B. Ljung, S. Mardh, S. Forsen and E. Thulin, *Nature, Lond.* **292**, 777 (1981).
37. K. C. Srivastava and K. K. Awasthi, *Prost. Leuk. Med.* **10**, 411 (1983).
38. C. A. Maniglia, G. Tudor, J. Gomez and A. C. Sartorelli, *Cancer Lett.* **16**, 253 (1982).
39. S. Thorens and C. Haeusler, *Eur. J. Pharmac.* **54**, 79 (1979).