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SUPPRESSION OF TUMOR METASTASIS BY ANGIOGENESIS INHIBITION

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ANGIOGENESIS IS THE FORMATION of new blood vessels. AGM 1470 is a synthetic nontoxic compound that inhibits endothelial cell proliferation, angiogenesis in vivo, and primary tumor growth.[1,2] Clinical and experimental evidence suggests that the ability of tumors to metastasize and to grow at a secondary site may be angiogenesis dependent.[3,4] We, therefore, tested the ability of AGM 1470 to inhibit tumor metastases in four in vivo model systems. To determine if the ability to suppress tumor metastasis is due to a direct effect on the tumor cell population or the vasculature, we also studied the effects of AGM 1470 on the growth and migration of tumor cells and endothelial cells in vitro.

METHODS

In vivo: Tumor cells (1×10^5) were injected into the tail vein of male C57BL mice (6–7 weeks old, n = 6) (Jackson Lab). Tumor cells used were M27 and H59 (derived from a Lewis lung carcinoma),[5] and B16F10 melanoma. Animals were killed 22 (M27 and H59) and 30 (B16F10) days after tail vein injection. AGM 1470 (Takeda Chemical Industries, Ltd) was diluted in normal saline solution and was administered subcutaneously at a dose of 30 mg/kg every other day. The first dose was given six hours after the tumor cells were injected into the tail vein. Control mice received an equivalent volume of saline solution. In another metastasis system, subcutaneous Lewis lung carcinoma tumors were resected from mice after reaching a tumor volume of 1,000 mm^3. AGM 1470 treatment began after

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Inhibition of tumor metastasis by AGM 1470

Tumor Line	Treatment	*n*	Surface Metastases†	*P* value (2-tail)	Lung Weight*†(g)	*P* value (2-tail)
M27 Lewis	AGM 1470	20	14±4	<.05	.18±.02	<.05
lung carcinoma	Saline	20	30±5		.45±.07	
H59 carcinoma	AGM 1470	13	45±6	<.05	.37±.04	<.05
	Saline	13	76±9		.60±.08	
B16 F10	AGM 1470	15	4±3	>.05	.18±.03	<.05
melanoma	Saline	15	11±6		.47±.15	
After LLC	AGM 1470	14	8±1	<.05	.22±.04	<.05
resection	Saline	15	13±2		.37±.04	

*Normal lung weight = 0.15±.01 g, n=36.
†Values of surface metastases and lung weight are mean ± SE.

resection of the primary tumor. These animals were killed 14 days later. In all metastases experiments, immediately after the mouse was killed, the lungs were weighed, and the surface metastases were counted under a dissecting microscope (12× magnification).

In vitro: The effects of AGM 1470 on the chemotaxis of endothelial cells and M27 Lewis lung carcinoma cells were investigated in the Boyden chamber assay. Tumor cells were stimulated with the elastin peptide val-gly-val-ala-pro-gly. Bovine capillary endothelial cells were stimulated with basic fibroblast growth factor. In the proliferation assays, M27 Lewis lung carcinoma cells were treated with AGM 1470, and cells were counted in a Coulter counter.

RESULTS

In vivo: AGM 1470 significantly inhibited pulmonary metastases generated from intravenous injection in three tumor lines and from a primary tumor (Table). Hematoxylin and eosin sections of representative sets of lungs from all experiments (n=22) demonstrated consistently smaller, essentially avascular tumors in the lungs of the AGM 1470 treated mice.

In vitro: Half maximal inhibition of migration of M27 Lewis lung carcinoma cells occurred at approximately 30 ng/ml of AGM 1470. Half maximal inhibition of migration of capillary endothelial cells occurred at approximately 100 pg/ml of AGM 1470. In proliferation studies, the M27 tumor cells were not inhibited up to a dose of 10 ug/ml.

DISCUSSION

These studies demonstrate that AGM 1470 suppresses tumor metastases and that this effect is mainly through the inhibition of angiogenesis. This conclusion is based on the observation that (1) the tumors in the lungs of the mice treated with AGM 1470 are smaller and essentially avascular compared with the tumors in the lungs of the saline-treated mice, (2)

small amounts of AGM 1470 are necessary to inhibit endothelial cell migration in vitro, and (3) previous in vitro and in vivo evidence that AGM 1470 is a potent angiogenesis inhibitor.[2,3] The ability of this compound to inhibit endothelial cell migration at a concentration three orders of magnitude less than is needed to inhibit tumor cell migration adds further evidence that AGM 1470's mechanism of inhibition is directed toward the tumor vasculature.

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ANTICATABOLIC EFFECT OF CIMATEROL IN VITRO IN TUMOR-BEARING ANIMALS

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CANCER-INDUCED CACHEXIA remains a potentially serious complication, in that the loss of greater than 20% of body protein may be fatal. The administration of supplemental nutrition is not a proven solution to cancer-induced malnutrition, since the tumor host does not maximally use the calories provided. Selective β_2-agonists have demonstrated significant anabolic properties.[1] The use of these agents in the tumor-bearing animal in conjunction with supplemental nutrition has resulted in a significant increase in lean body mass.[2] To determine which aspect of protein metabolism is affected by the β_2-agonists in tumor-bearing animals, an in vitro experiment using skeletal muscle was performed using the β_2-agonist, cimaterol.

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