Accumulation and Localization of Gallium-67 in Various Types of Primary Lung Carcinoma

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The uptake and location of Ga-67 were investigated in 15 primary pulmonary carcinomas. The accumulation in the tumor was determined by scintigraphy of the patient, grain counts over fields of tumor cells in autoradiographs of tumor-tissue samples, and gamma counts in specimens of the tumor. Good correlation was found between the results obtained with these three methods.

The relationship between accumulation of Ga-67 in the tumor and the histologic type of tumor was also studied. Undifferentiated carcinomas, and tumor cells in squamous-cell carcinomas showed significantly more Ga-67 than tumor cells in adenocarcinomas. No correlation was found between the presence of inflammatory infiltrates in or around the tumor and the grade of the scintigraphic images. In the autoradiograms, lymphocytes, plasma cells, granulocytes, and macrophages showed less radioactivity than the tumor cells—or none at all. Collagen fibers appeared to have bound some Ga-67, but necrotic areas showed no uptake.

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Gallium is known to concentrate in several types of malignant tumors (*1–4*), though not specifically. Several cell types involved in nonmalignant processes also concentrate Ga-67 to some extent (*1,3,5*). This tumor-seeking ability is now widely used for tumor visualization by scintiscanning. According to the literature, Ga-67 scintigrams of malignant neoplasms of the lung are almost always positive, whatever the histologic type of the tumor (*1,3,4*). Since in our experience Ga-67 scans of malignant lung tumors are sometimes negative, however, we attempted to determine whether there is any correlation between the Ga-67 uptake and the histologic type of the tumor. Because macrophages, lymphocytes, and granulocytes have also been reported to concentrate Ga-67 (*6–10*), we also investigated the possibility that accumulation of Ga-67 in these cells, during inflammatory infiltration around or within the tumor, might contribute to a positive scintigraphic image.

MATERIALS AND METHODS

Patients. Fourteen men and one woman, ranging in age from 49 to 77 yr, were selected for surgical resection treatment. In 14 of them bronchial carcinoma could be diagnosed preoperatively by cytologic or histologic examination; the remaining patient was merely suspected of having bronchial carcinoma. In all 15 cases the diagnosis was confirmed from the surgical specimens. The preoperative studies included Ga-67 scintigraphy. The interval between ligation of the blood vessels of the tumor during the operation and tissue sampling was less than 1 hr.

Scintigraphic methods. Ga-67 citrate solution was administered intravenously in doses ranging from 2 to 3 mCi. A large-field gamma camera, adjusted for 290 keV with a 20% window was used to obtain images 24 hr (or occasionally 48 hr) after administration of the radiotracer. The images were read as positive (+) or normal (−) according to the uptake of Ga-67 at the site of the lesion. If necessary, a second dose of Ga-67 citrate was administered 48 hr before the operation.

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Wet autoradiography. Three or four pieces of tissue (each approximately 3.5 mm^3) taken from central and peripheral parts of the tumor and the tissue directly adjacent were fixed for 2 hr in Bouin's fluid. The paraffin sections were cut 6 μm thick and the Kodak AR 10 stripping-film technique was employed.

Dry autoradiography Strumpf (*12*). Three or four pieces of tissue (each aproximately 1 mm^3), taken from various parts of the tumor and close to it, were immediately frozen in undercooled N_2. Sections were cut 2 μm thick, freeze-dried, and applied to reversed Kodak AR 10 stripping film on cover slips. The interval between the injection of Ga-67 and the beginning of exposure of the autoradiograms (wet as well as dry) was approximately 78 hr (the half-life of Ga-67). The exposure time for the autoradiograms was 3 wk. They were stained with haematoxylin and eosin.

Gamma counting. Small pieces of tissue (three to eight, each approximately 1 mm^3) taken adjacent to those used for autoradiography were counted in a gamma counter on the same day.

Grain counting in the autoradiograms. Because the grain density in the films of the autoradiograms was very low, making it impossible to determine the number of grains per cell, grains were counted in 100- by 100- μm squares over fields of tumor cells with the aid of an eyepiece provided wtih a grid. Fields of tumor cells with the fewest cells of other types were selected. Background grains were counted in squares just outside the sections and the count subtracted. When there were fields of other types of cells or tissues and these fields were sufficiently large and showed few cells of other types, grains were also counted in 100- by 100- μm squares in these areas. Grain counts over the contents of large blood vessels were rarely ever above background counts.

RESULTS AND CONCLUSIONS

Fifteen primary lung carcinomas were investigated. Table 1 gives the grain counts for tumor cells in the autoradiograms (wet technique) of tissue samples, gamma counts for tissue samples, and the scintigraphic results. These three categories show good correlation. The highest grain counts for tumor cells in autoradiograms and the highest gamma counts for tissue samples, occur in the group of undifferentiated and squamous-cell carcinomas, and the corresponding scintigraphic images are all positive. Adenocarcinomas have lower grain counts in autoradiograms and lower gamma counts in tissue samples, and two out of three have normal scintigraphic images. Two tumors were difficult to assign to either of these groups. One was a mixed adenocarcinomatous and squamous-cell carcinoma; the other an oat-cell tumor with low overall radioactivity but with several "hot spots" showing much higher radioactivity by autoradiograms. There are significant differences ($0.005 > Pd > 0.001$) between the grain counts over tumor cells in the autoradiograms for the group of ten undifferentiated and squamous-cell carcinomas and

TABLE 1. ACCUMULATION OF Ga-67 IN VARIOUS HISTOLOGIC TYPES OF PRIMARY LUNG CARCINOMA

Pulmonary carcinomas	Mean grain counts over tumor cells in autoradiograms (wet technique, squares of 100- × 100-μm) ± standard error of the mean	Scintigraphic image	Mean gamma counts in small tumor samples (cpm/mg wet tissue, 3–8 pieces)
Undifferentiated carcinoma	131.7 ± 28.9 (14)*	+	ND†
Poorly differentiated squamous-cell carcinoma	122.7 ± 22.1 (6)	+	ND
Moderately differentiated squamous-cell carcinoma	94.1 ± 12.8 (15)	+	ND
Poorly differentiated squamous-cell carcinoma	83.1 ± 6.0 (49)	+	316
Poorly differentiated squamous-cell carcinoma	73.6 ± 11.9 (11)	+	352
Moderately differentiated squamous-cell carcinoma	61.5 ± 8.4 (11)	+	ND
Undifferentiated carcinoma	53.2 ± 5.6 (9)	+	ND
Undifferentiated carcinoma	50.3 ± 5.4 (28)	+	ND
Poorly differentiated squamous-cell carcinoma	47.8 ± 5.1 (35)	+	ND
Well-differentiated squamous-cell carcinoma	45.6 ± 3.5 (41)	+	581
Adenocarcinoma	25.2 ± 3.2 (12)	+	120
Adenocarcinoma	20.8 ± 5.8 (6)	−	67
Adenocarcinoma	−6.9 ± 2.8 (22)	−	50
Moderately differentiated squamous-cell carcinoma with local adenocarcinomatous differentiation	3.4 ± 2.9 (8)	−	68
Oat-cell carcinoma (without focal radioactivity)‡	5.0 ± 3.5 (12)	+	155

* Values in parentheses give number of counts.
† ND = not determined.
‡ Several focal areas with increased radioactivity (fields of tumor cells with mean grain count of 69) were encountered.

the group of three adenocarcinomas.

These results point to a quantitative difference in uptake of Ga-67 between the squamous-cell and undifferentiated carcinomas on the one hand, and the adenocarcinomas on the other. Ramsdell and his coworkers (*13*) also found normal Ga-67 scans in patients with adenocarcinomas of the lung, and Rasker et al. (*14*) reported a smaller uptake of Co-55 bleomycine in adenocarcinomas than in other types of carcinomas of the lung. Possible differences between the metabolism of the cells of the adenocarcinomas and the group of other carcinomas, remain to be investigated.

Gallium-67 could be demonstrated in the tumor cells with both dry and wet autoradiographic techniques. We assume that the wet technique detects Ga-67 bound to proteins or protein-rich structures, whereas the dry technique also detects free Ga-67 ions (*15*). Comparison of the grain counts for these two types of autoradiograms is not possible, due to technical differences concerning, for instance, section thickness and the distance between section and film. However, since we did not find large differences in the number and localization of grains between the two methods, it seems likely that most of the Ga-67 present in tumor cells was bound to proteins. Grain counts were therefore made in "wet" autoradiograms all the more because different cell types could be recognized more easily in the paraffin sections than in the freeze-dried sections.

Most of the grains in the film were found over the cytoplasm of the tumor cells rather than over the nucleus. Since the resolution of Ga-67 in the wet autoradiograms is very good (*15*), it can be assumed that little or no protein-bound Ga-67 was present in the nuclei of the tumor cells.

In the tumors, cells other than tumor cells concentrate Ga-67. We found increased grain counts over fields of lymphocytes and plasma cells, and over fields of connective tissue, where the grains lay mainly over the collagen fibers. Too few of these grain counts could be done and they varied appreciably; nevertheless, they were hardly ever as high as the counts over tumor cells in the same tumor. Areas with many polymorphonuclear leucocytes did not have higher grain counts (a few observations). Accordingly, no correlation could be found between the scintigraphic images and the extent of inflammatory infiltration in or around the tumors. Fields of necrosis were normal, or sometimes showed a very few grains. Macrophages were almost always loaded with carbon pigment granules, which were difficult to distinguish from any grains in the overlying film in spite of the difference in level. We obtained the impression, however, that little or no bound Ga-67 was present in macrophages. Neither could appreciable amounts of soluble Ga-67 be detected in leucocytes and macrophages with dry autoradiography.

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