Immunoscintigraphy of Human Pancreatic Carcinoma in Nude Mice with F\((ab')_2\) Fragments of Monoclonal Antibodies to CA 19-9 and CEA


Radiolabeled monoclonal antibodies directed against tumor antigens have been used to detect tumors in animals [1–6] and men by gamma imaging. Many of these studies used antibodies or their fragments reactive against colon carcinomas [7–9], melanomas [10, 11], and mammary carcinomas [12]. Most of the localization studies were performed using I-131 labeled antibodies. The detection of the tumor, however, is highly dependent on the concentration and distribution of the antigen recognized by the antibody.

Clinical investigations have recently been carried out using 19-9, a monoclonal antibody that reacts specifically with human gastrointestinal cancer [13] and anti-CEA antibodies for radioimmunodetection of colon carcinoma [14, 15]. The antigens recognized by the two antibodies are shed into the circulation where their serum levels can be detected by radioimmunoassays and can be used as tumor markers for gastrointestinal cancer [16, 17].

In the present study iodinated monoclonal antibody fragments to CA 19-9 and CEA were evaluated for their ability to localize specifically in human pancreatic carcinoma xenografts hosted in nude mice by scintigraphy and tissue uptake measurements [18].

Materials and Methods

A human pancreatic adenocarcinoma was passaged by s.c. implantation of slices of tumor tissue (diameter 1.5 mm) into the right flank of nude mice. Mice bearing tumors 200–400 mg in weight were given an intravenous injection of 1.8 MBq of iodinated antibody fragments (specific activity 55 MBq/mg) approximately 5–6 weeks after implantation of the tumor. Thyroid uptake of free iodine was blocked by adding perchlorate to the drinking water 48 h before activity injection and throughout the experiment.

The study in nude mice was performed in three parts. Distribution, tumor uptake, and the whole body retention of a) 19-9 F\((ab')_2\) fragments and an unspecific IgG of the same subclass, b) 19-9 F\((ab')_2\) fragments and anti-CEA F\((ab')_2\) fragments, and, c) whole anti-CEA antibody and its F\((ab')_2\) and Fab fragments were compared.
In one group of animals the CA 19-9 levels in blood were detected by radioimmunoassays before and after injection of the 19-9-antibody.

The whole body clearance of the injected antibodies was determined by whole body counting of the animals immediately after injection of the activity (100% retention) up to 6 days. For scintigraphic imaging the animals were anesthetized by an intraperitoneal injection of 100 mg ketamine (Ketanest) and 10 mg xylazine (Rompun) per kg body weight. Serial scintiphotos were obtained 6 and 12 h after injection of the antibodies and subsequently every 24 h up to 6 days. Imaging studies were performed with a Philips gamma camera equipped with a 2 mm pinhole collimator. At least 30,000 counts per image were collected. Images were recorded from the I-131 peak (365 keV with a 20% window) and the data were stored with a digital computer into a $64 \times 64$ pixel matrix (Philips NPS with DEC PDP-11/34).

The biodistribution data were obtained by killing and dissecting animals in groups of at least 4 at 24, 48, 72, 96, and 144 h after injection. Blood, tumors, heart, lungs, liver, kidneys, spleen, stomach, large intestine, pancreas, thyroid, muscle, and femur (bone marrow) were removed and weighed. The radioactivity was measured in a gamma counter and expressed as percentage of injected dose per gram of tissue.

**Results**

The tissue distribution of 19-9 F(ab')$_2$ fragments at 1, 2, 4, and 6 days after activity injection compared to that of an unspecific IgG is shown in Fig. 1. The highest activity concentration for 19-9 was found in the tumor between 48 and 144 h post injection (p.i.). Only at 24 h p.i. the concentration in the blood was higher. The activity in blood declined from 2.5% per gram at 24 h to 0.08% at 96 h and 0.03% at 144 h.

The radioactivity in the tumor revealed a high variability in uptake. The mean value was found 2.1% per gram at 24 h and dropped to 0.6% at day 4 and 0.09% at day 6 p.i. Of all other tissues excised only the lung showed an activity concentration higher than that found for the blood. The faster clearance of radioactivity from the blood pool and normal tissue than from the tumor leads to increasing tumor-to-blood ratios over a 4-day period after activity injection. The maximum tumor-to-blood ratio was 7.8 (range 4.1−14.2) on day 4 p.i. and declined to 3 (range 2.4−6.0) at day 6 probably because of the release of radioactivity from the tumor.

Scintigraphic images obtained at 6 and 12 h after injection of I-131-labeled 19-9 F(ab'), fragments primarily showed the activity in the areas of heart and lungs (Fig. 2). At this early phase of the study a bladder activity was seen in many cases, too. Imaging permitted tumor detection at 12 h p.i., but optimum tumor localization was reached at day 4 after injection of the labeled antibody fragments. At that time almost only tumor radioactivity was seen, as the activity in the area of heart and lungs significantly decreased. The weight of the implanted tumor in Fig. 2 was 310 mg at dissection 96 h p.i. The tumor-to-blood ratio was 14.2:1.