Comparative Pharmacokinetics of a Murine Monoclonal Antibody to a Rat Colon Tumor in Rats and Nude Mice

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Abstract

The pharmacokinetics of the 125I-labeled murine anti-rat colon carcinoma monoclonal antibody, E4, and its F(ab')2 fragments were compared in normal Sprague-Dawley rats as well as in syngeneic BDIX rats and nude mice bearing the tumor to which the monoclonal antibodies had been generated. 125I-labeled irrelevant antibody of the same IgG2a subclass or its labeled F(ab')2 fragments were used as controls. Results of labeled antibody uptake after i.v. administration were analyzed in terms of accumulation and localization indices for normal tissues and tumor. Whole E4, which is tumor specific by immunoperoxidase staining, bound to a variety of normal tissues, in addition to the tumor. These tissues included liver, stomach, colon, and lung of rats bearing s.c. tumors. Targeting to the tumor was better in rats bearing i.p. tumors, but targeting to other organs was also high. The use of F(ab')2 fragments in rats demonstrated a lack of correlation between in vitro tissue uptake of injected murine monoclonals, or their fragments, and in vitro histochemical studies. In contrast to observations in rats, tumor alone was specifically targeted with whole E4 or F(ab')2 fragments in tumor-bearing nude mice. Trichloroacetic acid precipitation of rat and mouse tissue radioactivity indicated that labeled antibody and, not catabolites or free iodine, was being measured. These data suggest that studies on the xenogeneic nude mouse model may not necessarily be relevant to the choice of monoclonal antibodies for clinical diagnostic imaging or therapy.

Introduction

With the advent of MoAbs,2 radioimmunodetection of tumors has been widely implemented clinically, with some reports of promising initial results (1–5). Most of the experimental data which support therapeutic trials leading to clinical use of mouse monoclonal antibodies are based on in vitro analysis of their tissue reactivity and on biodistribution studies in tumor-engrafted nude mice (6–8). The behavior of a mouse monoclonal antibody in a syngeneic host bearing a xenogeneic tumor may, however, be different from its behavior in a xenogeneic host. Therefore, we compared pharmacokinetics of a mouse monoclonal antibody against rat colon carcinoma in tumor-bearing rats or nude mice. We also studied the pharmacokinetic of F(ab')2 fragments, based on reports that the use of monoclonal antibody fragments improves the localization of xenogeneic tumors engrafted into hamsters (9) and nude mice (10–13) and considerations that the presence of Fc receptor-positive cells in several tissues could have some role in modifying tissue targeting.

Materials and Methods

Animals. BDIX rats were purchased from Centre National de la Recherche Scientifique (Orleans, France) and housed and bred at the NIH animal facility. Animals were 3 to 6 months old when used. Sprague-Dawley rats and nude mice (6 to 12 weeks old) were obtained from the NIH. Animals were provided ad libitum with NIH rat and mouse ration (NIH-07) and tap water and kept in individual cages. Three days before injection of radiolabeled antibodies, animals were provided with 2% potassium iodide-enriched water, in order to saturate their thyroid with cold iodine.

Cell Lines. Rat colon adenocarcinoma DHD K12 TRb (TR), was generously provided by F. Martin (Dijon, France) (14). Cells were cultured in RPMI 1640 (Gibco, Grand Island, NY), enriched with 10% fetal bovine serum (Hyclone, Logan, UT) and 1% penicillin-streptomycin (Gibco). To engraft animals and to maintain cells in culture, cells were detached from flasks by a 1-min incubation with a solution of EDTA disodium salt (1 mg/ml; Sigma, St. Louis, MO) followed by 3 min of incubation with 1 mg/ml trypsin (Sigma) in Ca2+/Mg2+-free Hanks' balanced salt solution (Gibco).

Monoclonal Antibodies. The murine monoclonal anti-colon carcinoma antibody used in this study, E4, as well as its in vitro reactivity against normal rat tissues has been described previously (15). The isotype-matched IgG2a mouse MoAb, 17.1A (kindly provided by Centocor, Inc., Malver, PA) was used as a control. 17.1A does not react histochemically with the rat tumor or with normal rat or mouse tissues. F(ab')2 fragments of MoAb E4 and 17.1A were produced by treatment with pepsin and purified by Bionetics Research (Rockville, MD). Monoclonal antibodies and their F(ab')2 fragments were radiolabeled with 125I (E4) or 125I (17.1A) as described previously (16). The specific activity varied between 1 and 2.5 µCi/µg.

Pharmacokinetic Studies. In each experiment, 107 or 5 × 106 TR cells were injected s.c. into 9 BDIX rats or 9 nude mice, respectively. In another independent experiment, 107 TR cells were injected i.p. into 9 BDIX rats. Ten days later, s.c. tumors had reached 170 ± 19 and 43 ± 12 mg (mean ± SEM) for rats and mice, respectively. Intraperitoneal tumor gave a great tumoral mass in the omentum and multiple metastases through the abdominal wall and on different organs. At this point, 50 µg (rat) or 10 µg (mouse) of 125I-E4 and the same amount of 125I-17.1A were injected i.v. into anesthetized animals. The same procedures were performed in the case of antibody fragments. One, 3, or 5 days after injection, 3 animals were anesthetized and blood samples were drawn by cardiac puncture (rats) or eye bleeding (mice). Animals were sacrificed by cervical dislocation, and the organs were resected, washed in phosphate-buffered saline, and carefully blotted dry. Organs were weighted and the radioactivities of 125I and 125I determined in a gamma counter (1218 Compugamma; LKB, Bromma, Sweden). In the rat studies, one animal of the day 5 group was placed in a metabolic cage and its urine was recovered on days 1, 3, and 5. The 125I and 125I radioactivities of a 1-ml aliquot were counted.

Data Analysis. Results are expressed by calculating two different parameters. First, the AI is defined as the ratio between the percentage of the injected dose per g found in each tissue and the dilution factor, equivalent to the theoretical value of the percentage of injected dose that would be found in tissues if the dose injected were identically distributed in the animal’s whole body. This value is 100/body weight (g). The AI allows comparison of antibody uptake by the tissues of animals of different weights. In addition, the AI is an indication of the avidity of a tissue for a given antibody. The AI is less than 1 in tissues in which antibody uptake is not significantly different from the uptake due to the dilution of the antibody through the body, considering that some antibody is eliminated through the urine. An AI of 1 or greater in a given tissue means that the antibody, regardless of its antigen specificity, is accumulated in that tissue. The second parameter is the localization index, first described by Moshakis et al. (17) and defined as

1 Presented at the "Second Conference on Radioimmunodetection and Radioimmunotherapy of Cancer," September 8–10, 1988, Princeton, NJ.
2 The abbreviations used are: MoAb, monoclonal antibody; TR, trypsin resistant; AI, accumulation index; LI, localization index.

873s
PHARMACOKINETICS OF ANTITUMOR MOAB IN RATS AND NUDE MICE

Localization index = \[
\frac{\% \text{ dose/g relevant antibody (tissue)}}{\% \text{ dose/g control antibody (tissue)}} \\
= \frac{\% \text{ dose/g relevant antibody (blood)}}{\% \text{ dose/g control antibody (blood)}}
\]

This parameter is a measure of the specificity of the antibody localization and, as for the accumulation index, is independent of the animal's weight.

Trichloroacetic Acid Precipitation. To ensure that the radioactivity found in the tissues was due to antibody and not to its catabolites or free iodine, several organs from one animal of each day studied were homogenized in a solution of 20% trichloroacetic acid in water. The homogenized organs were centrifuged at 3000 x g for 15 min and the precipitate and supernatant radioactivities were counted.

Results

Pharmacokinetics of E4 Antibody and F(ab')2 Fragments. Greater than 95% of the radioactivity taken up by rat and mouse tissues was precipitable with trichloroacetic acid, indicating that no catabolites or free iodine were being measured (data not shown). The pharmacokinetics of whole E4 in BDIX rats was studied in two different situations, after s.c. or i.p. injection of TR cells. The accumulation indices calculated for s.c. and i.p. tumors are presented in Figs. 1a and 3a, respectively. Most organs show similar E4 and 17.1A AI values in both cases; the exceptions are lung for E4 and tumor for 17.1A. These differences were more clearly reflected by the localization indices (Figs. 2a and 3d). LI values of all rat tissues were greater than 1 in both cases, indicating a specific uptake of E4 as compared with 17.1A. Lung and tumor showed higher localization indices in the case of i.p. injection of tumor cells. This result seems to indicate a better accessibility of the antibody to the tumor. Nevertheless, this increased accessibility does not significantly improve the localization of tumor with respect to other organs. E4 showed similar biodistribution in tumor-bearing BDIX and normal Sprague Dawley rats (data not shown), indicating that the results obtained are not dependent on a particular strain or model.

Nude mice showed E4 AI values which were similar for most organs, except blood, spleen, and tumor (Fig. 1b). However, LIs reflect a large difference between nude mice and rats in E4 biodistribution. For all normal mouse tissues, LIs were around 1 or slightly lower (Fig. 2b), indicating no discrimination between E4 and 17.1A uptake by these tissues. On the other hand, tumor LIs were higher than 1 on days 1 and 3, reflecting specific targeting to the tumor. However, on day 5, the LI dropped below 1, indicating no prolonged targeting of the antibody at the tumor site.

The accumulation indices found for F(ab'), E4 in BDIX rats and nude mice (Fig. 1c, and d) were around 10 times less than those of the whole antibody, indicating faster blood clearance of fragments. Blood distributions of E4 and 17.1A fragments in rats were more similar than those of the whole antibodies. Remaining tissues showed some differences between the AI values of E4 and 17.1A fragments, with generally greater values for E4. In nude mice, however, tumor was the only tissue showing a much higher AI value for F(ab'), E4 than for F(ab'), 17.1A.

The differences observed at the level of Als became more evident when the localization indices were examined. In BDIX rats (Fig. 2c), muscle, colon, stomach, heart, lung, and brain showed high LIs. Tumor showed LIs similar to that of liver, although greater than the rest of the tissues studied. This situation was not very different from that found in the case of the whole antibody for the s.c. injection of TR cells (Fig. 2a). In contrast, in the case of the nude mice (Fig. 2d), the LIs found for E4 were about 1 in all tissues, with tumor the only tissue which showed LIs greater than 1.

Discussion

The pharmacokinetic studies reported here point up important considerations for the use of xenogeneic monoclonal antibodies in a syngeneic host-tumor system. An anti-rat colon carcinoma antibody localized exclusively in the rat tumor transplanted in nude mice. When injected into normal rats or rats bearing the tumor to which the antibodies had been raised, the
antibody was taken up by a variety of normal tissues, as well as by the tumor.

_in vitro_ studies showed that E4 reacted only with tumor tissue (15). However, no clear correlation could be established between E4 immunoperoxidase reactivity and _in vivo_ binding. E4 injected into rats was targeted to several normal tissues, including liver, colon, stomach, and lung, in addition to tumor. The targeting to the tumor was improved in the case of i.p. injection of tumor cells, but E4 targeting to the other tissues remained significant as compared to the tumor. For example, liver, which was negative by immunoperoxidase, retained as much antibody as lung, which was only slightly positive _in vitro_ (15). Several reasons may explain this behavior, including differences in organ vascularization and accessibility of the antibody to tissues expressing even small amounts of antigen. The increased targeting obtained with i.p. tumors in rats, which are more vascularized than s.c. tumors, supports this hypothesis.

Probably as a consequence of E4 binding to several rat tissues, major differences between it and the control were seen in the blood; the irrelevant antibody was more slowly eliminated. This fact can influence the localization index, since the localization index actually corresponds to the division of tissue/blood ratio for specific antibody by the same ratio for nonspecific antibody. Differences in blood values will affect the localization index greatly and can artificially increase the values in some organs, even those binding very few antibody molecules. For example, brain tissue did not bind E4 _in vitro_ and did not retain E4 in excess of nonspecific antibody _in vivo_, but LI values were clearly above 1, indicating specificity of targeting. Despite this consideration, the extremely elevated LIs indicate clearly that specific E4 targeting to rat tissues occurs.

Data in the nude mouse showed similar biodistribution of both specific and control antibodies in blood and other normal tissues. The engrafted rat tumor is the exception, actually the only tissue expressing rat species-associated antigens. This gave a LI value of 1 for all tissues except the tumor, which showed
higher LI values and, in consequence, was the only tissue specifically targeted by the antibody. In the case of mice, the localization index seems to be meaningful and LIs >1 useful because no antigenic competition between other tissues occurs.

The pharmacokinetics of F(\(\text{ab}'\))\(_2\); E4 fragments showed several differences from that of the whole antibody. First, the injection of antibody fragments yielded more similar blood distributions of specific and nonspecific antibody in both rats and mice. The second important difference was the much faster clearance of the fragments as compared with the whole antibody. This fact has been reported previously in normal and nude mice (18, 19). Accumulation indices are about 10 times lower for the fragments than for the whole antibody in all organs of both BDIX rats and nude mice.

The use of F(\(\text{ab}'\))\(_2\); E4 fragments increased the tumor localization indices of engrafted nude mice and did not modify the localization indices of the other organs, which remained around 1. In this model, therefore, the use of antibody fragments actually improved the specificity of tumor targeting, as reported for human colon carcinomas (11, 12), melanomas (13, 20), gliomas (21), and tumors expressing placental alkaline phosphatase (22). However, a similar event did not occur in rats, the injection of F(\(\text{ab}'\))\(_2\); E4 did not greatly modify the localization indices obtained with the whole antibody in normal rat tissues, and the tumor LI was even smaller in that case.

Our results indicate that conclusions drawn from a xenogeneic tumor system regarding the biodistribution of tumor-specific murine monoclonal antibodies do not necessarily apply to syngeneic models. The situation found in our rat model may be comparable to that of patients with colorectal carcinoma who have been given injections of monoclonal antibodies selected by using the nude mouse model. Our results strongly support the idea that other animal models, more appropriate to each clinical situation and tumor type, are needed in order to obtain clinically pertinent information to the selection of monoclonal antibodies as candidates for imaging and therapy in humans.

References

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