SELECTIVE ACCUMULATION OF MONOCLONAL ANTIBODIES IN THE LUNGS AFTER INJECTION OF CYCLOPHOSPHAMIDE INTO RATS

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The possibility of targeted transport of active drugs to particular organs and tissues has been studied for a long time. Much of the research into targeted transport of various compounds, including radioisotopes, has been undertaken with antibodies against surface antigens [1, 2]. However, Welt and co-workers used an intracellular antigen of an experimental mouse tumor as the target [3]. These workers describe a high degree of antibody accumulation in the tumor, although it took them more than 2 months to eliminate the background radioactivity and to obtain high-quality gamma-scintigrams. Such a long time interval between injection of labeled antibodies and the obtaining of high-contrast images does not satisfy clinical requirements. However, if procedures aimed at increasing the number of antigen-binding sites accessible for binding with antibodies are carried out beforehand, for example, if small doses of cytostatics causing death of the most sensitive cell clones are injected, this might perhaps solve the problem. High selectivity of isotope delivery to zones of necrosis and necrobiosis ought to be ensured in this case by a low concentration of intracellular antigens in the normal tissues and a higher concentration of antibody-binding sites in the target tissues. Selective irradiation (when alpha- or beta-sources are used) of intact tumor cells, located close to antibody binding sites, may ensure a dose of radioactivity sufficient to produce regression of the tumor.

In the investigation described below, cyclophosphamide was used as model of damaging agent, for it undergoes metabolic activation actually in the tumor tissues (through the action of intracellular phosphatases), it has a contact action, and it is sufficiently effective when given by local administration during chemotherapy of malignant tumors [1, 2].

EXPERIMENTAL METHOD

Male Wistar rats weighing initially 200-250 g were used. Cyclophosphamide was injected into the caudal vein in a dose of 15, 45, or 60 mg/kg body weight in 0.5 ml physiological saline. The control animals received 0.5 ml of physiological saline alone. On the 3rd day after injection of cyclophosphamide, ^{125}I-labeled 2C5 monoclonal antibodies were injected into rats of the first group, and IIID3 monoclonal antibodies into those of the second group. Immunoglobulins from the total fraction of mouse serum were used as control for nonspecific uptake. Each group (including the control) contained 10 animals. The quantity of protein injected was 20 μg and the specific radioactivity

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TABLE 1. Biodistribution of Labeled Antibodies after Injection of Cyclophosphamide into Rats (ratio of radioactivity: organ/blood)

Dose of cyclophosphamide, mg/kg	Liver	Spleen	Kidneys	Lungs
2C5				
—	0,80±0,04	0,43±0,02	0,40±0,03	0,90±0,05
15	0,92±0,02	0,57±0,03	0,45±0,05	1,30±0,06
45	0,97±0,01	0,66±0,07	0,47±0,05	1,90±0,20
60	1,12±0,05	0,85±0,05	0,47±0,03	4,11±0,30
IIID3				
—	0,61±0,10	1,88±0,40	0,83±0,20	0,57±0,09
15	0,70±0,08	2,14±0,30	0,99±0,19	0,57±0,10
45	0,69±0,10	2,05±0,20	1,01±0,11	0,55±0,09
60	0,71±0,10	1,99±0,20	1,12±0,13	0,56±0,07
Nonspecific IgG				
—	0,68±0,09	0,48±0,10	0,52±0,08	0,54±0,16
15	0,81±0,11	0,52±0,07	0,49±0,04	0,61±0,08
45	0,95±0,15	0,59±0,03	0,47±0,10	0,59±0,10
60	0,98±0,07	0,76±0,11	0,53±0,09	0,64±0,11

of the samples 0.2 μCi/ml. The animals were decapitated under ether anesthesia, blood samples and biopsy specimens from the organs were taken after 4 h, and accumulated radioactivity was determined on a "Compugamma" counter. The parameter of evaluation was the ratio of accumulated radioactivity: organ/blood.

EXPERIMENTAL RESULTS

The biodistribution of two clones of monoclonal antibodies was studied: IIID3 – antibodies reacting with cytoplasmic vimentin Intermediate filaments, and 2C5 – antibodies reacting with cell nuclei. Table 1 gives the results of distribution of these antibodies and of nonspecific immunoglobulins (mean ± standard deviation), labeled with ^{125}I, among the rats' organs, depending on the injected dose of cyclophosphamide. Two factors demand attention.

1. The ratio of radioactivity of spleen/blood is quite high after injection of labeled IIID3 antibodies, and independent of the dose of cyclophosphamide injected beforehand. Probably this active uptake of these antibodies, which belong to the IgM class, may be explained on the grounds that about 30% of all platelets having receptors for Fc-sites of immunoglobulins on their surface, are located in the spleen; the latter are sufficiently numerous, for the IIID3 molecule is a pentamer and, consequently, for one antibody there are five Fc-sites. The existence of any other specificity of these monoclonal antibodies for spleen cells remains an open question, for they (IIID3) have not been adequately studied.

2. So far as 2C5 monoclonal antibodies are concerned, the character of dependence of their accumulation in the lungs on the injected dose of cyclophosphamide is evident. Unfortunately, the nuclear component which is the antigen for 2C5 has not been identified, and it is therefore difficult to speak of the mechanism of "damage" to the cell taking place under the influence of cyclophosphamide, although we know that it cross-links DNA strands [3]. The absence of a similar dependence for IIID3 suggests that changes and, perhaps also, damage to the integrity of the cell membrane, are not sufficiently serious to allow passage of the quite large IgM molecule. In studies of the toxic action of this compound, the development of hepatic and renal pathology is mentioned [6] and, what is particularly important for us in connection with the aims of the present investigation, the appearance of necrotic changes in lung tissue [5]. We did not carry out a histologic investigation of the lungs of the rats receiving cyclophosphamide. However, the appearance of petechial and massive hemorrhages in them, the presence of small abscesses, and a

change of color of the lung tissue were noted visually. This evidently suggests with a high level of probability that this compound can be used as model of an agent damaging lung tissue cells in order to guarantee access of antibodies to nuclear antigenic structures.

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