Immunotoxicological Characteristics of Preparations Based on Carcinoembryonic Antigen and Mucin Containing CA 125 Antigen

S. M. Sitdikova, B. S. Amandzholov, V. Yu. Sel'chuk, N. G. Kormosh, K. P. Laktionov, Yu. S. Lebedin,* S. V. Chukanov,* A. Yu. Toptygin,* L. V. Manzyuk, and F. V. Donenko

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 129, No. 4, pp. 462-464, April, 2000
Original article submitted July 26, 1999

Experiments on male hybrid mice demonstrated that specific immunotherapy with preparations based on carcinoembryonal antigen and mucin containing CA 125 antigen was not associated with general toxicity, local irritating effect, and hepatorenal dysfunction. The absence of toxicity is apparently due to the fact that antigens injected intramuscularly or subcutaneously virtually do not enter the blood. Injections of preparations based on carcinoembryonal antigen and mucin containing CA 125 antigen to mice induced a standard immune response with predominance of class M immunoglobulins during the early terms and class G immunoglobulins at later terms.

Key Words: *carcinoembryonal antigen; CA 125 antigen; specific immunotherapy; toxicity; immune response kinetics*

Specific immunotherapy of some malignant tumors are now actively developed. Preparations based on tumor cells and tumor-associated antigens are called vaccines in foreign literature, and the therapeutic method is called antitumor vaccination, but in view of principal differences from classical vaccination we consider that the term "specific immunotherapy" is more adequate. The efficiency of specific immunotherapy with human carcinoembryonic antigen (CEA) in mice with tumors containing CEA gene was previously demonstrated [1,2]. Clinical trials of CEA-based preparations are in progress.

Here we studied some immunotoxicological characteristics of original Russian preparations based on CEA and mucin containing CA 125 glycoprotein, an antigen expressed in the majority of patients with ovarian and breast cancer. The preparations were prepared from native antigens isolated from tumor nodes by affinity chromatography. Toxic and side effects and pharmacokinetics of CEA and CA 125 glycoprotein, and the kinetics of immune response were studied in experimental animals.

MATERIALS AND METHODS

Male hybrid mice aged 2-3 months were used. For evaluation of the pharmacokinetics of CEA and CA 125, the preparations were injected intraperitoneally, subcutaneously, and intramuscularly in a dose of 1 mg/mouse. Blood was collected 5, 15, 30, 60, and 180 min after challenge with the antigen.

The kinetics of immune response was evaluated after single subcutaneous injection of CEA and CA 125 in a dose of 100 μg/mouse and antibody titer was measured on days 10 and 17 after challenge by enzyme immunoassay using Roche kits.

N. N. Blokhin Cancer Research Center, Russian Academy of Medical Sciences; *Hema-Medika Research-and-Production Company, Moscow

0007-4888/00/0004-389$25.00

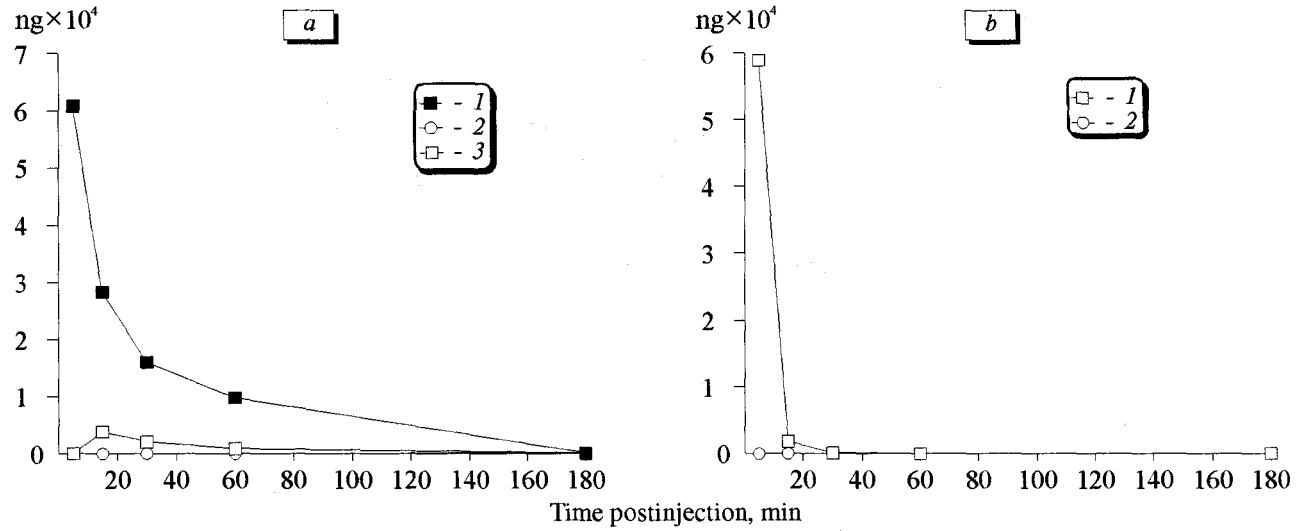

Fig. 1. Pharmacokinetics of carcinoembryonal antigen (*a*) and CA 125 antigen (*b*) after intraperitoneal (*1*), subcutaneous (*2*), and intramuscular (*3*) injection.

Biochemical studies were carried out on a Cobas-mira biochemical analyzer (Roche) with standard kits. Concentrations of test substances are presented in μM.

Toxic effect of CEA and CA 125 was evaluated by changes in body weight and standard biochemical parameters of the blood in animals treated with the antigen in comparison with controls. The animals were immunized with CEA and CA 125 by injecting 10-fold immune doses (up to 1 mg/mouse) on days 1, 2, 3, 10, 17, and 24. Blood was collected on the next day after the last injection.

Each group consisted of 10 animals. The data were statistically processed using Fisher—Student test, the differences were considered significant at $p<0.05$.

RESULTS

The half-life of CEA in the organism after intraperitoneal injection was about 15 min, and after 180 min no drug was detected in the peripheral blood (Fig. 1, *a*). After intramuscular injection of CEA-based drug negligible amounts of the antigen were detected in the plasma, with the maximum at the 15th min. After subcutaneous injection, CEA was not detected in the peripheral blood.

Mucin containing CA 125 was completely eliminated from the peripheral blood 15 min after intraperitoneal injection and was not detected during 180 min after subcutaneous injection (Fig. 1, *b*).

The mice receiving CEA-based preparation and mucin containing CA 125 developed a standard immune response with predominance of IgM at the beginning (up to days 12-13) and IgG at later terms (Fig. 2).

No side effects were observed after injection of CEA-based preparation and mucin containing CA 125:

TABLE 1. Effect of Specific Immunotherapy with CEA-Based Preparation and Mucin Containing CA 125 on Body Weight in Mice (*M±m*)

Time after immunization, days	Control	CA 125	CEA
0	21.0±0.2	21.0±0.3	21.0±0.3
2	24.2±0.3	24.3±0.4	24.5±0.5
9	25.7±0.6	26.1±0.7	26.6±0.7
16	26.9±0.6	27.1±0.7	27.9±0.8
18	27.1±0.7	27.4±0.7	28.2±0.6
30	29.8±0.8	30.1±0.7	30.2±0.8

Note. Here and in Table 2: all differences from the control are significant.

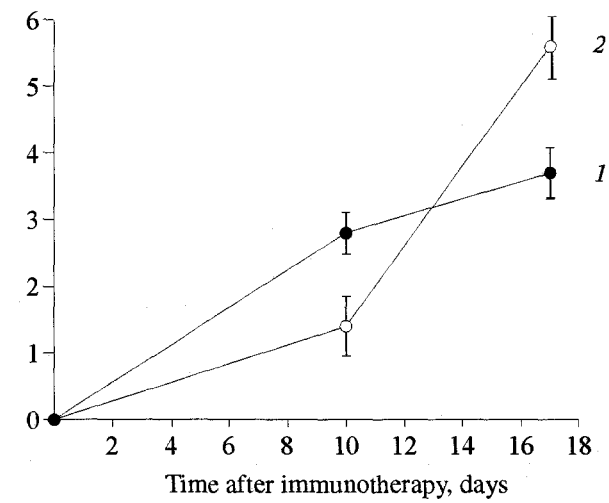

Fig. 2. Time course of IgM (*1*) and IgG (*2*) after specific immunotherapy with preparation based on carcinoembryonal antigen and mucin containing CA 125. Ordinate: arb. units.

TABLE 2. Effect of Immunization with Mucins Containing CEA or CA 125 on Blood Biochemistry in Mice (*M±m*)

Parameter	Control	CEA	CA 125
Total bilirubin	4.54±0.80	3.87±0.27	3.86±0.65
Direct bilirubin	1.90±0.10	1.94±0.13	1.94±0.12
Creatinine	36.34±2.73	27.88±4.38	29.71±2.60
ALT	35.86±1.57	37.11±2.99	33.50±2.88
AST	226.03±18.56	169.13±17.38	169.51±27.22
Creatine kinase	2568.57±867.36	1188.29±144.94	2409.71±652.87

the time course of body weight (Table 1) and biochemical parameters of the blood (Table 2) did not differ from the control. No cases of animal death or irritation at the site of injection of doses surpassing the therapeutic doses were observed.

Hence, CEA-based preparations and mucin containing CA 125 even in concentrations far surpassing immune doses caused no symptoms of general intoxication, local reactions, and exerted no negative effects on the hepatorenal functions, thus meeting the requirements to drugs for specific immunotherapy. Such low toxicity is apparently due to the fact that the antigens injected subcutaneously or intramuscularly virtually do not enter the circulation. The results obtained in animal experiments do not allow us to predict the immunogenic reaction to CA 125 and CEA in humans. Studies of immunogenicity of these agents and identification of their antigenic determinants the response to which will correlate with the therapeutic effect in cancer patients is the task of the first phase of clinical trials.

It is noteworthy that original Russian drugs based on CEA and mucin containing CA 125 are native highly glycosylated proteins. The use of recombinant CEA obtained by gene engineering methods does not answer the question on the role of glycosylated antigens in the development of antitumor immunity, though this may explain the differences in the efficiency of immunization with CEA in experimental animals and in patients [1].

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