IMMUNOENZYME DETECTION OF SPECIFIC BRAIN ANTIGENS AS A TEST OF PERMEABILITY OF THE RAT BLOOD-BRAIN BARRIER AFTER ACUTE γ-RAY IRRADIATION

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Disturbances of function of the blood-brain barrier (BBB) after acute radiation damage have been the subject of numerous investigations [1, 3, 5, 9]. Permeability of BBB, as one of its functions, has so far been studied only in the blood–brain direction. This approach is not without advantages because of the impossibility of ruling out an independent effect of certain substances not only on the BBB, but also on structures in the CNS which regulate its permeability. Meanwhile the question of the use of specific brain substances to evaluate the functional state of the BBB has not been adequately studied [2, 8].

The object of this investigation was an immunoenzyme study of changes in permeability of BBB for cytoplasmic antigens of the rat brain in the early stages after acute γ-ray irradiation.

EXPERIMENTAL METHOD

Experiments were carried out on 200 noninbred rats of both sexes (average weight 150-220 g), subjected to a single whole-body irradiation on the "Luch" (^{60}Co) apparatus in doses of 1 to 10 Gy, and with a dose rate of 0.96 Gy/min. Blood serum of the control and irradiated rats served as the test object. The permeability of BBB for specific brain α_2-globulin (α_2M) [12] and for acid gliofibrillar antigen [6] was investigated by an immunoenzyme method [7]. Antisera to specific α_2M and to acid gliofibrillar antigen (GFAP) were obtained by immunizing chinchilla rabbits with purified preparations of these antigens, isolated by methods described in [12] and [6] respectively. Antibodies to the above antigens were isolated from monospecific antisera on immunosorbents prepared on the basis of CNBr–sepharose 4B (Pharmacia Fine Chemicals, Sweden) and of purified preparations of α_2M and GFAP by the method in [13]. The concentration of immunoglobulins in the fraction of isolated antibodies was determined by Ouchterlony's double immunodiffusion method, using donkey antiserum against rabbit immunoglobulins (Boehringwerke, West Germany). The concentration of specific antibodies to α_2M and GFAP was determined by double immunodiffusion in the modification in [4]. For these investigations we used the "sandwich" version of the immunoenzyme method suggested in [11]. The polystyrene plates were activated with a 0.0005% solution of antibodies to α_2M and a 0.002% solution of antibodies to GFAP in 0.05 M carbonate buffer, pH 9.6, for 20 h. During preparation of the conjugate of antibodies to brain antigens with the enzyme, horseradish peroxidase (type VI, from Sigma, USA) was used. The antibodies were conjugated with peroxidase by the periodate method [10]. A 0.08% solution of 5-aminosalicylic acid was used as the substrate. The results were recorded on a Titerteck Multiscan multichannel spectrophotometer (Flow Laboratories, England).

EXPERIMENTAL RESULTS

The immunoenzyme system for detection of α_2M in biological fluids, developed in the course of the experiment, enabled it to be detected within concentrations of between 0.8 and 102.0 ng/ml. Optimal working of the system was observed when polystyrene plates were activated by the antibodies in a concentration of 5 μg/ml and with a 1:50 dilution of the

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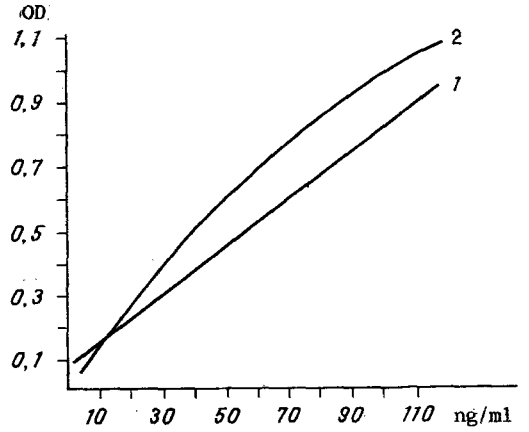

Fig. 1. Graph showing dependence of concentration of specific brain antigens on optical density at 420 nm. Abscissa, concentration of brain antigens (in ng/ml); ordinate, absorbance at 420 nm. 1) Calibration curve for GFAP; 2) calibration curve for α_2M.

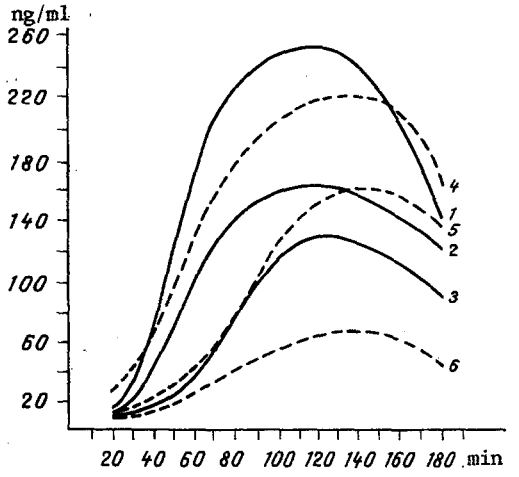

Fig. 2. Graph showing dependence of concentration of specific brain antigens in rats' blood serum on time after acute γ-ray irradiation in doses of 10, 5, and 1 Gy. Abscissa, time after acute γ-ray irradiation; ordinate, concentrations of specific brain proteins (in ng/ml). 1) Curve showing dependence for GFAP during irradiation in a dose of 10 Gy; 2) the same, for a dose of 5 Gy; 3) the same for a dose of 1 Gy; 4) curve showing dependence for α_2M with irradiation in a dose of 10 Gy; 5) the same for a dose of 5 Gy; 6) the same for a dose of 1 Gy.

conjugate. The immunoenzyme system for GFAP analysis was capable of detecting it between concentrations of 1 and 128 ng/ml. Optimal working of this system was achieved by the use of antibodies in a concentration of 20 μg/ml to activate the polystyrene plates, and with dilution of the antibody – enzyme conjugate of 1:60. Typical curves showing dependence of the concentration of α_2M and GFAP in the test sample on optical density at 420 nm, plotted from the results of 50 experiments, are given in Fig. 1. Statistical analysis of the standard deviation of the points, which did not exceed 1.15%, confirmed the reliability and good reproducibility of the systems developed.

We used the immunoenzyme method of detection of α_2M and GFAP to study the permeability of BBB in rats of the control group and also in animals exposed to acute γ-ray irradiation.

The blood serum of rats of the control group (200 animals) contained α_2M in a concentration of not more than 7 ng/ml and GFAP, in a concentration of 4 ng/ml. These values were taken as the upper limits of normal for the above-mentioned antigens in rat blood serum.

Immunoenzyme analysis of specific α_2M and GFAP in the blood serum of irradiated rats demonstrated the effect of an increase in permeability of BBB for these antigens; moreover, the rate of appearance of α_2M and GFAP, and also their concentrations correlated with the dose of irradiation (Fig. 2). Irradiation of rats with a dose of 10 Gy led to an increase in the concentration of GFAP and α_2M as early as 20 min after the end of irradiation. However, the GFAP concentration under these circumstances was 15-20 ng/ml, whereas the α_2M level was rather higher, namely 25-30 ng/ml. Later the character of the curves changed in different ways: the serum GFAP concentration rose rapidly to reach a maximum after 2 h, whereas the α_2M concentration rose more slowly and its peak level was rather lower. An increase in the doses of irradiation led both to a decrease in the rate of appearance of brain antigens in the blood and to a considerable fall of their maximal levels – from 250 to 90 ng/ml for GFAP and from 220 to 45 ng/ml for α_2M. The sharp fall in the concentrations of both GFAP and α_2M 3 h after the end of irradiation (to 50%) confirms the existence in mammals of active mechanisms for maintaining homeostasis in brain tissue, based on highly developed self-regulation of the BBB permeability systems.

It is interesting to note that antigens expressed by different cells (α_2M – a marker of oligodendrogliocytes, GFAP – a marker of astrocytic proteins) differ in their ability to pass through the blood-brain barrier, although their molecular weights are about equal. The possibility cannot be ruled out that this effect is due to a difference in the hydrophobic properties of the proteins.

Thus not only can the use of the immunoenzyme method for the detection of markers of glial cells (GFAP and α_2M) reveal changes in the permeability of BBB in the early stages after acute γ-ray irradiation, but the study of specific brain antigens can create a basis for the development of criteria for the analysis of certain stages of metabolism in brain cells for which they are markers.

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