ELABORATION OF A RADIOIMMUNOANALYTICAL METHOD FOR THE DETERMINATION OF SERUM FERRITIN

A. GLAGOLIČOVÁ, V. WERNISCHOVÁ

Institute of Biophysics and Institute of Nuclear Medicine, First Medical Faculty, Charles University, Prague (Czechoslovakia)

(Received August 15, 1991)

A radioimmunoanalytical (RIA) method was elaborated for the determination of ferritin in human blood serum in clinical practice. Placental ferritin separated from the human after-delivery placental and antibodies against the human placental ferritin obtained by the immunization of rabbits with this antigen were used. The whole complex of basic conditions and parameters of the RIA method was tested including the estimation of the region of normal values and clinical tests. The method elaborated was compared with the commercial kit Ferritin RIA Amersham, code IM 1051, chosen as reference kit. The results of the determination of control parameters as well as ferritin levels obtained by the method elaborated exert good agreement with the reference kit and correspond to requirements for routine RIA practice.

Introduction

In current medical practice, a great variety of commercial RIA and IRMA kits is being routinely used for determining many substances. Further kits are being developed in spite of a considerable competition of non-isotopic methods based on similar principles. The advantage of all these in vitro methods is a high sensitivity in their analytical use. Ferritin is one of the substances interesting for clinical practice from the standpoint of its level in plasma and serum, depending on the amount of iron stored in the organism. A connection was also demonstrated with the damage to tissues in certain hematological and cancer diseases. The commercial kits for the determination of blood serum ferritin level are based on the use of spleen or liver ferritin and antibodies against them. Placental ferritin is a further available and suitable tissue ferritin. This ferritin and antibodies against the placental ferritin of our own production was used in the present work for the elaboration of our modification of the radioimmunoanalytical determination of blood serum ferritin level.
The elaboration of the RIA method for its analytical use was preceded by a standardization of human placental ferritin as well as of rabbit antibodies against human placental ferritin. The ferritin standardization was carried out by a gravimetric method, by a RIA determination with the help of the commercial Ferritin RIA Kit Amersham, and by spectrophotometric measurements at 280 nm. On the basis of comparing the results, the extinction coefficient was established as $\varepsilon = 125$, which was further used for determining the concentrations in particular batches of ferritin. The concentration calibration curve was plotted from absorbance values at 280 nm for known concentrations of spleen ferritin Calbiochem (1 mg/ml) in a range of 10 to 300 $\mu$g per ml of physiological saline. This calibration curve was employed to determine placental ferritin concentrations at different dilutions. Isolated and purified ferritin, used for the preparation of standards, for iodination, as well as for the RIA determination itself, was stored in PBS containing 0.1% of NaN$_3$ in sterile ampoules at 4 °C. The purity of isolated and purified ferritin was checked by electrophoresis on polyacrylamide (PAGE method).

The measurements performed in physiological saline medium involve errors due to adsorption with increasing dilution. In the case of a concentration of 300 mg/ml, the adsorption starts to be negligible (up to 5%) and the spectrophotometric analyses agree with results of RIA determinations, where the possibility of adsorption was abolished by a subsequent dilution by a medium containing bovine serum albumin (BSA).

The gamma-globulin fraction of antibodies used for the RIA was prepared from a mixture of selected antisera obtained by the immunization of rabbits by human placental ferritin. The necessary condition for the determination of the binding capacity of antibodies and also for elaborating a complete radioimmunoanalytical method (RIA) of the ferritin determination in the blood serum is the elaboration of a suitable separation technique for the isolation of the antigen-antibody complex. The use of Antibody II in combination with polyethyleneglycol (PEG 6000) is considered as the most frequently used separation technique. In our work, we chose available pig Antibody II against rabbit IgG (SwaRG – USOL Praha) and investigated the dependence of specific and non-specific bonds on the composition of the separation agent, dependence of incubation II on time (15, 30, 60, 120, min – 24 hours), on temperature (room temperature or – 4 °C) and on the separation agent volume (0.5, 1.0, 1.5 ml). The precipitation activity of the separation agent was also checked with the help of the commercial Ferritin RIA kit Amersham.

The specificity of antibodies against placental ferritin was compared with the help of the same commercial kit, which contains spleen ferritin as a standard. By the non-kit RIA method to be developed, cross reactions of antibodies against placental ferritin