AN IMPROVED ENZYME-LINKED IMMUNOSORBENT ASSAY FOR MEASURING PLASMA ERYTHROPOIETIN LEVELS IN THE RAT.

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To investigate the possible role of erythropoietin (EPO) in the pathogenesis of a drug-induced red cell hypoplasia in the rat, a sandwich enzyme-linked immunosorbent assay (ELISA) was validated to measure plasma EPO levels in that species. As the gene coding for EPO is highly conserved among species, a kit designated to quantify EPO in human plasma was selected, using rabbit polyclonal antibody against HuEPO and mouse monoclonal detection antibody conjugated with peroxidase (Roche). The standard procedure was improved by increasing the sample volume to 40 µL to achieve a higher sensitivity of the test (basal plasma concentrations low in young rats). Using this modified condition, the detection limit was 0.24 mU/mL and the quantitation limits were 1.5 to 100 mU/mL. EPO plasma levels were established on untreated Wistar rats (32/sex, 6-7 weeks old) and on 20%-bled Wistar rats (6/sex, 6-7 weeks old) to check the timing and the amplitude of the EPO release in response to an acute anemia. Plasma EPO was measured before, 4, 24 and 48 hr after bleeding. Normal EPO levels were 2.1 ± 0.7 and 1.7 ± 0.5 mU/mL in males and females, respectively. As expected, plasma EPO concentrations reached a peak 24 hr after bleeding (6- to 12-fold the normal values in females and males, respectively), reflecting the increase in erythroid activity. The selected test provides a rapid (3.5 hr), reliable and nonradioactive immunologic method for quantitating normal or elevated plasma EPO levels in the rat.

Mots-clé: Erythropoietin; Rat; ELISA