ERYTHROPOIETIN SECRETION DURING HYPOBARIC HYPOXIA. MODULATION OF THE RESPONSE BY THE LEVEL OF ERYTHROPOIESIS

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RESUMEN La eritropoyetina (EP0) es una hormona glucoproteica que forma parte de un mecanismo de retroalimentación negativa involucrado en el control de la eritropoyesis. La concentración de EP0 en plasma (pEP0) en los mamíferos depende del balance existente entre el aporte de oxígeno (0_2) a los tejidos y la demanda del gas por ellos. Sin embargo, observaciones clínicas y experimentales indican que otros factores, además del 0_2, estarían también involucrados en la modulación de la síntesis de la hormona. Por lo tanto, modelos experimentales murinos fueron desarrollados en nuestro laboratorio para investigar la relación posible entre la producción de EP0 0_2-dependiente y el nivel de la eritropoyesis. Esta fue estimulada mediante administración de rh-EP0 o inhibida mediante inyección de ciclofosfamida. Cuando ratones así tratados, junto a ratones controles, fueron expuestos durante 6 horas a hipoxia hipobárica para inducción de hipoxemia, pEP0 fue 208 % y 33 % del valor normal en los animales con eritropoyesis deprimida o estimulada, respectivamente. El catabolismo de EP0 no fue afectado por ninguno de los tratamientos. Estos resultados sugieren que la síntesis de EP0 en los mamíferos no sólo guarda relación con el balance entre oferta y demanda de 0_2 tisulares (estímulo principal) sino también con la actividad eritropoyética de la médula ósea (acción moduladora).

Palabras Claves : Eritropoyetina, Hipoxia, Hipobaria

RÉSUMÉ: Sécrétion d'érythropoïétine au cours de l'hypoxie hypobare. Modulation de la réponse par le niveau d'érythropoïèse.

L'érythropoïétine (EPO) est une hormone glycoprotéique faisant partie d'un mécanisme de rétroalimentation impliqué dans le contrôle de la production de globules rouges. Les niveaux plasmatiques d'EPO (pEPO) des mammifères sont liés à l'apport d'oxygène (O_2) aux tissus en fonction de leurs besoins. Les observations cliniques et expérimentales indiquent cependant que d'autres facteurs en plus de O_2 peuvent également être impliqués dans la modulation de la synthèse d'EPO. Des modèles expérimentaux de souris ont donc été développés dans notre laboratoire afin de rechercher une éventuelle relation entre la production d'EPO dépendante d'O_2 et le niveau d'érythropoïèse. Celle-ci a été stimulée par rh-EPO ou inhibée par cyclophosphamide. Les souris ainsi traitées et un groupe de souris normales ont été soumises à l'hypoxie hypobare pendant 6 heures. Chez les souris hypoxiques les valeurs de pEPO ont atteint 208 % par rapport à la normale chez les souris à l'érythropoïèse déprimée et 33 % chez celles où elle a été stimulée. Le catabolisme de l'EPO n'a pas été affecté par les traitements. On suggère donc que la synthèse de l'EPO chez les mammifères est liée non seulement à l'apport d'oxygène aux tissus selon leurs besoins (stimulus principal), mais aussi à l'activité érythroïde de la moëlle osseuse (action modulatrice).

Mots-clés : Erythropoïétine, Hypoxie, Hypobarie.

SUMMARY: Erythropoietin (EP0) is a glycoprotein hormone that is part of a feedback mechanism involved in the control of red cell production. Plasma EP0 levels (PEPO) of mammals are related to the oxygen (0_2) supply to tissues relative to their 0_2 needs. However, clinical and experimental observations indicate that factors other than 0_2 could be involved also in the modulation of EP0 synthesis. Therefore, experimental mouse models were developed in our laboratory to investigate the possible relationship between 0_2 dependent EP0 production and the level of erythropoiesis. Erythropoiesis was either stimulated by rh-EP0 or depressed by cyclophosphamide. When mice so treated, as well as normal mice, were made hypoxemic by a 6 hour-exposure to hypobaria, pEP0 was 208 % of normal and 33 % of normal in mice with depressed or stimulated erythropoiesis, respectively. EP0 catabolism was not affected by treatments. It is thus suggested that EP0 synthesis in mammals is not only related to the 0_2 supply to tissues relative to their 0_2 needs (main stimulus) but also to the erythroid activity of the bone marrow (modulatory action).

Key Words : Erythropoietin, Hypoxia, Hypobaria

Oxygen (0_2) transport within the body is often divided into a convective portion, comprising bulk transport of 0_2 to the capillaries, and a diffusive portion, consisting of off-loading of 0_2 within the capillaries and diffusion ultimately to cytochrome aa3 within the mitochondrion (1). Convective 0_2 transport (COT) is the product of blood flow and arterial 0_2 content (CaO_2), CaO_2 is a function of hemoglobin (Hb) concentration, oxyHB binding properties, and arterial $P0_2$ (PaO_2) The circulating red cell mass (RCM) is an organ that collaborates in COT by providing a HB mass that binds 0_2 is a reversible form. The organ is composed of red blood cells (RBC), which have a finite life span and lack the ability for self-renewal. The RCM is maintained at an optimal size for its function by adjustments in the rate of erythropoiesis since the red cell life span is a biological constant.

Erythropoiesis is thus a vital process that is able to adjust to different physiological and pathological conditions. Consequently, the level of erythropoiesis will decrease if the RCM is artificially increased by transfusion, or will increase under conditions that induce hypoxia, as defined by a diminished 0_2-carrying capacity of blood (anemia), by a decreased arterial $P0_2$ (hypoxemia), or by an increased HB 0_2-affinity at sea level.

Physiologic adjustments of the rate of erythropoiesis and RCM are mediated by erythropoietin (EPO), a glycoprotein which acts as a specific growth factor for erythroid progenitor cells in the bone marrow (2). The hormone is thus a part of a feedback mechanism involved in the control of erythropoiesis. It is mainly secreted by renal endocrine cells in inverse correlation with COT through the expression of an EPO gene apparently in response to both contitutive and hypoxia-induced transcription-regulating factors (3).

According to the almost 40-year old hypothesis of Fried et al (4) that EPO synthesis depends on the convective 0_2 supply to tissues relative to their 0_2 needs, it seems evident that the EPO production rate (EPO-PR) is negatively correlated to 0_2 availability, namely tissue $P0_2$. A structure, possibly a hemoprotein, has thus been proposed that senses the 0_2 tension and initiates a signal that turns on the expression of the EPO gene (5). According to this model, a kidney 0_2 sensor measures intersticial $P0_2$ and modulates EPO-PR by the kidney, which in turn adjusts the rate of erythropoiesis to meet the demand for 0_2-carrying cells.

Although Fried's hypothesis has received strong experimental and clinical support, the following evidences militates against its inherent simplicity, suggesting that factors other than 0_2 could modulate EPO synthesis:

1) Fried et al (6) reported that plasma EPO levels (pEPO) of WWw mice, which have a mild, congenital anemia, a decreased response to EPO, and a defect in their multipotential hematopoietic stem cells (HSC), were higher that those of comparably anemic non-mutants (+++). This difference was not longer present 7 days after transplanting marrow cells into WWv mice. At this time, the response of WWw mice to EPO was comparable to that of +++; yet the colonizing ability of their HSC was still defective. From these data, the authors suggested that EPO-PR at any level of anemia is modified by the ability of the hematopoietic cells to respond to EPO.

2) Barceló and Bozini (7) presented evidence that pEPO during continuous exposure to hypobaria in mice with marrow aplasia induced by whole body X-irradiation or 5-fluorouracil injection were higher than in control mice similarly exposed. These finding gave support to the hypothesis that a relationship exists between EPO-PR and the erythroid responsiveness to EPO.

3) Birgegard et al (8) measured pEPO in 23 patients before, during and after intensive cytostatic treatment courses for acute leukemia or before bone marrow transplantation. A marked increase was seen in all patients, starting 1 or 2 days after initiation of treatment. A peak was reached after about 7 days, after which pEPO fell rapidly, even in patients who were anemic at that time. In 13 of the patients there was no fall in HB levels that could explain the increase in pEPO. The increase was too large to be explained by an altered EPO metabolism or marrow utilization. Authors suggested the existence of a mechanism other than anemia for EPO-PR stimulation.

4) Piroso, Erslev and Caro (9) performed serial pEPO measurements in 6 patients with acute leukemia treated by intensive chemotherapy. In all cases pEPO increased after the onset of treatment, although the HB concentration remained at stable values. Subsequently pEPO gradually returned to baseline levels at the time of bone marrow recovery. It was concluded that this inapropriate increase in pEPO could be related to a direct or indirect effect of a suppressed marrow on sites of EPO production or catabolism.

5) Jelkmann and Wiedemann (10) compared pEPO in nonrenal anemic patients with erythrocytic hypoplasia or active erythropoiesis. In both groups, a negative correlation was determined between the blood HB concentration and the logarithm of pEPO. However, the two regression lines were not identical, and pEPO was significantly higher for the degree of anemia in the patients with erythroid hypoplasia. This data support the idea that, independent of the 0_2 offer, the proliferating erythrocytic progenitors lower pEPO by negative feedback.

In the five described studies pEPO was unexpectedly higher in humans or animals with demonstrated or assumed poor erythroid response to EPO. It was also assumed that pEPO reflects EPO-PR.EPO concentration in the plasma compartment, however, depends on the balance between EPO formation and EPO disappearance rates. Consequently, the above findings could be attributed to changes in either EPO synthesis or EPO catabolism, or both. To clarify this point, studies were performed in our laboratory (11, 12] to estimate both pEPO (during stimulation of hormone production by hypobaric hypoxemia] and plasma EPO half-life in mice in which the rate of erythropoiesis (RBSPR) was either

increased by rh-EPO administration or depressed by cyclophosphamide (CP) treatment. Two mouse models were thus developed in which the 0_2-carrying capacity of blood, pEPO, blood viscosity, 0_2 supply/demand ratio - factors that alter EPO production - and the kinetics of plasma EPO were within normal values in spite of intense stimulation or depression of erythropoiesis. Any observed difference in pEPO in response to the hypoxemic stimulus between experimental and control mice should therefore be attributable to the increased EPO-PR. As expected from previously reported results (13), hypoxiadependent EPO-PR was inversely related to the level of erythropoiesis occurring in the animals during exposure to hypobaric hypoxia, EPO-PR being 208% of normal in mice with CP-induced depression of erythropoiesis and 33% of normal in those with EPO-induced enhancement of erythropoiesis.

No evidences exist on the nature of the operating mechanism. However, data suggest that a functional link could exists between the EPO-responsive cells in the bone marrow and the EPO-synthesizing cells that could modulate the hypoxiadependent expression of the EPO gene. If this is really the case, then the EPO-PR in mammals will be not only related to the 0_2 supply to the tissues relative to their 0_2 needs (main stimulus) but also to the erythroid activity of the marrow (modulatory action).

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