66

CLINICAL APPLICATIONS OF ERYTHROPOIETIN(EPO) IN PEDIATRICS.

Elena F.Morshchakova,Irina P.Kukushkina,A.Pavlov
Ryazan Branch of the Institute of Pediatric Hematology,Ryazan,Russia.

Cytokines are decisive for the regulation of the immune system as well as the renewal and maturation of the hemopoietic cells.The most important groups of these factors,several of which are already produced by gene technology,are interferons,interleukines and hemopoietic growth factors. The only real hormone among them is EPO.With the development of recombinant human EPO(rhEPO) it was possible to manipulate erythropoiesis in a positive fashion independently of endogenous EPO production.Because EPO is a hormone and its production is regulated at the level of its gene independently of age,gender or plasma concentration,the availability of sensitive and specific immunoassay provided an opportunity to define clinical situations in which anemia was associated with EPO deficiency.rhEPO can be regarded as a form of hormone replacement therapy,which is very effective in correcting the anemia of chronic renal failure in children.rhEPO allows transfusion-dependent dialysis children to become independent of red blood cell transfusions. rhEPO is currently used in the next conditions: (1)renal anemia in children with preterminal chronic renal insufficiency,(2)anemia in preterm infants(using rhEPO it has been shown a clear reduction in the requirements for blood transfusions in preterm infants),(3)anemia associated with chronic inflammation,infection or cancer, (4)anemic cancer children on combination chemotherapy,(5)myelodysplastic syndromes,multiple myeloma,pure erythroid aplasia,sickle cell disease,(6)in allogenic bone marrow transplantation.

67

MECHANISMS OF ERYTHROPOIETIN ACTION

Anatol D. Pavlov, Elena F. Morshchakova
Ryazan Branch of the Institute of Pediatric Hematology, Ryazan, Russia

Erythropoietin(EPO),the obligatory growth factor for erythroid cells is a specific molecular messenger between erythropoietic(hypoxic)stimuli and EPO-responsive cells(ERC)in bone marrow. EPO has the most restricted range of target cell activity among the other hemopoietic growth factors.EPO normally appears have three major functions:(1)it maintains the proliferation pool of committed erythroid progenitors(ECP),(2)permits their differentiation and(3)recruits immature ECP(presumably not in cell cycle)into Mature ECP pool(ERC).The proliferation and maturation of erythron also are controlled by EPO.No evidence that EPO interacts with any cells other than ECP which contain both high-affinity(300 copies, kd=0.09 nM)and low-affinity(600 copies,kd=0.5&nM) receptors for it.In cells expressing both high- and low-affinity receptors,only the high-affinity form might be responsible for the biologic effect of EPO,as few low-affinity receptors are expected to be occupied at physiologic concentrations of EPO.The hormone generates signals for both differentiation and proliferation of ECP.This dual action can be dissected by treatment with herbimycin,which selectively inhibits the proliferative activity of EPO.Unfortunately,EPO-induced differentiation has been difficult to analyze at the molecular level.The earliest effect of EPO on target cells is an increase in the synthesis of all classes RNA(mRNA,rRNA and tRNA).The transcription of globin mRNA is controlled,presumably, certain modifications of chromosomal proteins (acetylation and phosphorylation of histones and non-histones).

68

PROSTAGLANDINS AND LEUKOTRIENES MODULATE PROLIFERATION OF NORMAL AND TRANSFORMED BLOOD CELLS.

C. Haberl, L. Hültner, M. Falk, D. Egger, S. Geuenich, K. Dittmann, W. Wilmanns and C. Denzlinger
Medizinische Klinik III, Klinikum Grosshadern, Ludwig-Maximilians-Universität, and Forschungszentrum für Umwelt und Gesundheit, D-81377 München

Prostaglandins (PG) and leukotrienes (LT) are potent lipid mediators involved in inflammatory and allergic processes. Moreover these mediators have been shown to modulate proliferation of different cell types. We studied the effects of prostaglandins and leukotrienes and their pharmacological inhibitors on the proliferation of normal and transformed blood cells. Supplementary analytical studies were performed in mast cells, which are rich sources of these lipid mediators.

Proliferation of cells was estimated using a colorimetric assay (MTT-test). LTC4, LTB4, PGD2, PGJ2 and delta-12-PGJ2 were detected in serum-free suspensions of murine bone marrow-derived mast cells (from Balb/c mice) by combined use of high performance-liquid chromatography and radioimmunoassay.

PGD2 and its metabolites, PGJ2 and delta-12-PGJ2, inhibited in micromolar concentrations the growth of HL-60 cells, different types of lymphoma cells and mast cells. Indomethacin had no effect on proliferation in these in vitro models. Neither LTC4 nor LTB4 modulated the growth of myeloic precursors. The instability of these leukotrienes may have prevented an effect on cell proliferation. In contrast, the leukotriene synthesis inhibitors AA-861 and MK-886 reduced the proliferation of HL-60 and lymphoma cell-lines. However, these drugs had no effect on the proliferation of mast cells. PGD2 and its metabolites, LTB4 und LTC4 were produced by murine mast cells in significant amounts.

These results suggest that prostaglandins and leukotrienes play a role as growth-modulatory signaling systems in normal and transformed blood cells.

69

EFFECTS OF BLEOMYCIN ON LEUKOTRIENE PRODUCTION IN MAST CELLS IN VITRO AND IN PATIENTS IN VIVO.

S. Geuenich, D. Egger, L. Hültner, C. Haberl, M. Humann, W. Wilmanns, and C. Denzlinger
Medizinische Klinik III, Klinikum Grosshadern, Ludwig-Maximilians-Universität, and Forschungszentrum für Umwelt und Gesundheit, D-81377 München

Bleomycin is a widely used antitumor antibiotic. The major side effects of the drug are allergic and inflammatory reactions, including severe pneumonitis.

We investigated whether bleomycin has an effect on the production of leukotrienes, potent lipid mediators, which have been shown to induce several key symptoms of allergy and inflammation such as plasma extravasation, smooth muscle contraction and activation of leukocytes. Leukotriene production was determined in murine bone-marrow-derived mast cells in the presence and absence of calcium ionophore and bleomycin. In addition, patients suffering from low-grade non-Hodgkin's lymphoma were investigated who were treated with bleomycin (15 mg, intravenously) on day 14 of the COP-BLAM regimen.

Leukotrienes in the cell supernatants were determined by radioimmunoassay. Leukotriene production in patients was assessed by determining leukotriene E4 and N-acetyl-leukotriene E4 in urine by means of combined high-performance liquid chromatography and radioimmunoassay. These leukotriene metabolites represent established indicators of leukotriene production in vivo.

Bleomycin increased leukotriene production in the mast cell system in a dose-dependent way. This effect was not observed with several other antineoplastic agents. In patients, bleomycin induced a distinct increase in the endogenous leukotriene production. This effect was most pronounced in a patient suffering from major inflammatory adverse reactions.

Our data indicate that some of the inflammatory side effects of bleomycin may be mediated by leukotrienes.