Erythropoietic effects of PGE_2 and 2 endoperoxide analogs[1]

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Summary. The erythropoietic effects in exhypoxic polycythemic mice of two endoperoxide analogs were assessed and compared with PGE_2. The 9α,11α epoxymethano analog (U-44069) was found to be a much more potent erythropoietic stimulus than the 11α,9α analog (U-46619) or PGE_2.

The role of the renal prostaglandins (PG) in modulating kidney function has been the subject of intensive investigations in the past few years. Recently, a new role for the renal PG in the control of production of the renal glycoprotein hormone erythropoietin (Ep) has been demonstrated. We have previously found that arachidonic acid[5] and PGE_2 and PGA_2, but not $PGF_{2\alpha}$, had an Ep-dependent erythropoietic effect in exhypoxic polycythemic mice[6]. In addition, arachidonic acid and PGE_2 were found to cause the release of Ep in the isolated programmed posthypoxic perfused canine kidney[5,6]. Because the bicyclic PG endoperoxides are obligate intermediates in the conversion of arachidonic to PGE_2, the present studies were undertaken to determine the erythropoietic effects of two methanoepoxy analogs of the prostaglandin endoperoxide PGG_2.

Materials and methods. The erythropoietic effects of the endoperoxide analogs (EPA) and PGE_2 were determined in HAM/ICR strain virgin female mice (22–25 g) who were made polycythemic by daily exposure over 2 weeks to 22 h-periods at 0.42 at of pressure in a chamber. On days 1, 2, 3, 4 and 5 following their removal from the hypobaric chamber, the mice were injected (s.c.) with saline, 0.1 U human urinary Ep standardized against the International Reference Preparation (IRP-B), or 100, 200, 400 and 800 µg/kg of the respective PG. On posthypoxic day 8, each mouse received 0.5 µCi of radioactive iron (^{59}Fe) via the tail vein. 2 days later, posthypoxic day 10, the mice were exsanguinated by cardiac puncture, and 0.5 ml of blood per mouse was withdrawn and counted on a solid scintillation counter. The percentage of ^{59}Fe incorporated into newly formed red blood cells (RBC) was determined. 5–6 mice were injected with each sample per group and the mean 48 h ^{59}Fe incorporation into RBC were used to determine the erythropoietic activity of the PG. All mice with hematocrits below 51% were discarded from the assay. Student's 2-tailed-t-test was used for the statistical analyses. The following PG[4] were used in the study: a) PGE_2; b) U-44069, 9α,11α-epoxymethano analog; and c) U-46619, 11α,9α-epoxymethano analog. The PG were dissolved in absolute ethanol and diluted to 10% with glass-distilled water, producing a final concentration of 1 mg/ml and stored at −76 °C until used. They were diluted immediately prior to their injection into the mice.

Results. As illustrated in figure 1, we found that there were distinct differences between the erythropoietic activity of the 2 structurally similar analogs. The dose-response regression line for the 9α,11α-analog was found to be highly significant ($r=0.96$ and $p<0.001$) with as little as 200 µg/kg, possessing erythropoietic activity comparable to 0.1 U Ep. In contrast, the other analog 11α,9α-epoxymethano did not demonstrate a dose-response relationship ($r=0.74$ and $p>0.4$) though it should be noted that it was not possible to test the highest dose 800 µg/kg because of its very potent constrictor activity. In contrast, though diminished in response relative to the 9α,11α-analog (figure 2), PGE_2 was found to stimulate erythropoiesis in a dose-dependent manner ($r=0.92$ and $p<0.01$). The erythropoietic activity of the 9α,11α-analog was found to be 1.5–3.7 times more potent than PGE_2 in causing a dose-related stimulation of erythropoietic activity in exhypoxic polycythemic mice.

Discussion. The existence of an endoperoxide intermediate in PG biosynthesis was first postulated by Samuelsson in 1965[7]. Because the naturally occuring endoperoxides have a half-life of about 4–5 min in vitro[8], it was necessary to study the erythropoietic effects of endoperoxides in vivo with stable endoperoxide analogs (EPA) that had a methylene group substitution for the peroxide oxygen at C-9 and C-11 respectively. A problem, of course, with an analog of a naturally occuring substance is that the dose-response relationship may be different from the natural product. We did, however, observe that the C-11 methylene substituted EPA (U-44069) did show a dose-dependent stimulation of

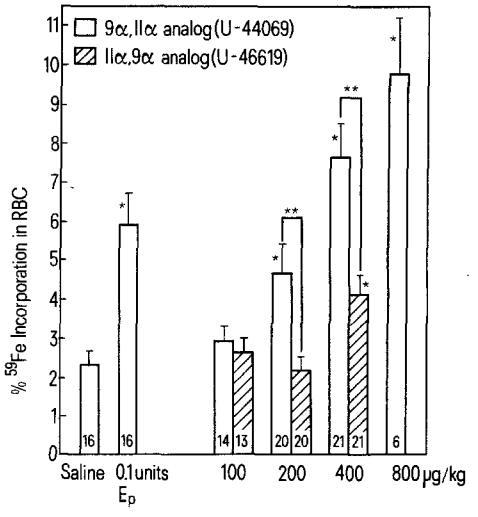

Fig. 1. % of ^{59}Fe incorporation into RBC of exhypoxic polycythemic mice injected with the EPA U-44069 and U-46619. Each bar is the mean ± SEM; at the bottom of each bar is the number of mice. * Significantly different from saline ($p<0.05$). ** Significant difference between each group at each dose ($p<0.05$).

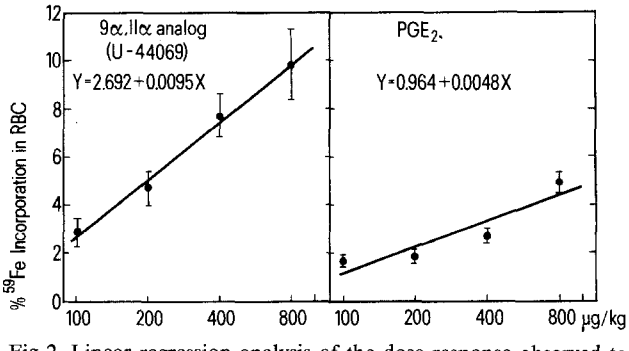

Fig. 2. Linear regression analysis of the dose-response observed to 9α,11α-EPA and PGE_2 in exhypoxic polycythemic mice. Each point is the mean ± SEM.

erythropoiesis, an effect which was lacking in the mice treated with the 11α,9α-EPA substituted at C-9. In fact it was very difficult to evaluate the 11α,9α-EPA due to its very potent smooth muscle constrictor activity causing respiratory problems in the mice. The 9α,11α-EPA was also found to be more potent than naturally occuring PGE_2. The 9α,11α-EPA has been shown in other biological systems to primarily mimic the effects of PG-endoperoxides, whereas the 11α,9α-EPA has been found to mimic the effects of thromboxane A_2. Therefore, the erythropoietic effects of the EPA appear to be related to the endoperoxide-like activity rather than the constrictor activity associated with thromboxanes which might produce renal ischemia and enhance Ep production from a hypoxic kidney.

It is of interest that all the PG we evaluated in vivo for its ability to stimulate erythropoiesis possessed oxygen at the C-9 position. It therefore appears that a structure-activity-relationship probably exists, with regard to the ability of PG to interact with a potential PG-receptor which modulates Ep production and hence erythropoiesis. This appears to be confirmed by the observation that PG having some other group at position C-9, e.g., hydroxyl ($PGF_{2\alpha}$) or methyl (11α,9α-EPA) apparently lack the ability to stimulate in vivo erythropoiesis.

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Haemopoietic stem cell concentration and CFUs in DNA synthesis in bone marrow from different bone regions

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Summary. The concentration of colony-forming cells (CFUs) is about 40% less in sternal marrow than in the marrow of lumbar vertebrae and femora. Marrow of trabecular bones in lumbar vertebrae contains fewer mitotically active CFUs than marrow of trabecular bones in the femoral distal epiphysis and metaphysis, or the peripheral marrow near the cortical bone in the femoral diaphysis. Only a minor part of the variability of the results in the CFU-assay is due to differences in CFU-concentrations between individual donor mice; pooling of the cell suspensions does not substantially decrease variability. Specific pathogen-free mice yield the same results as BALB/c mice from conventional breeding.

Colony-forming stem cells (CFUs) are primitive haemopoietic cells with a multiple differentiation capacity[2] and capable of extensive self-replication[3]. The macrophage - granulocyte committed stem-cells (CFUc) have a restricted differentiation capacity, a high mitotic activity[4] and little or no self-replicative ability[5]. Both populations show a high radiosensitivity[2,6]. It has been suggested that colony-forming cells may be accumulated rather near the endosteal surfaces[7-9], although not all experiments confirm this[10,11]. Localization of CFUs in bone marrow is important, e.g. when histogenesis of haemopoietic cells or radiotoxic effects from short-range bone-seekers on the stem cells are studied. The concentrations of CFUs in marrow regions of selected cortical and trabecular bones as well as in a marrow cell population not close to the bone surface, are described. The proportion of CFUs in DNA synthesis is also measured; and an analysis of different variance components is used to distinguish between the individual variability of host mice and other experimental errors.

Materials and methods. Experiments were performed with BALB/c mice from conventional breeding and with specific pathogen free (SPF) mice, both from S.C.K.-C.E.N. breeding. 5 male 3-month-old mice were used per observation. They were killed by cervical transection; the right femur, 4 lumbar vertebrae and the sternum were removed and the outsides thoroughly cleaned. Trabecular bone from epiphysis and metaphysis at the distal and proximal end of the femur were separated from the cortical bone of the diaphysis, with a miniature saw-file. The axial marrow of each diaphysis was flushed out twice with 1 ml of α-MEM (Flow Laboratories, UK) under a constant flushing pressure of 250 g and collected in a plastic tube, yielding a cell population not close to the endosteum. Microscope sections of the flushed bones showed that peripheral marrow remained and that central marrow was absent. Nucleated cells remaining in the peripheral shafts, as well as those of the lumbar vertebrae, of the sterna and of the distal femoral ends, were obtained by grinding the bones separately in a mortar and then washing them in a fixed volume of α-MEM. To estimate individual variability, the marrow suspensions from each mouse were kept separately. In other experiments the cells of 5 donor mice were pooled for each bone marrow region. After homogenization of the cell suspensions and haemolysis of red blood cells, the cell concentration was measured with an electronic counter (Coulter counter ZF). The suspensions were preserved in plastic tubes under aseptic conditions on melting ice and each was assayed for CFUs and CFUc concentrations. Each experiment included, as a control, the femoral marrow from 3 3-month-old male BALB/c mice, prepared by grinding the whole, cleaned right femora. In each experiment all the data were normalized to that of the femoral control cell suspension.

CFUs concentrations were determined using the spleen colony assay technique[2] and CFUc were assayed by culturing in a single-layer soft agar system. Complete medium consisted of α-MEM, 0.22% $NaHCO_3$, gentamicin (100 µg/ml α-MEM, Shering Corp., USA), 10% fetal calf serum and 10% horse serum (Flow Laboratories, UK). Colony formation was stimulated by conditioned serum[12] derived from