

In vitro effect of dexamethasone phosphate on hematopoietic progenitor cells in preterm infants

Paola Papoff, Robert D Christensen, Jenny Harcum, Yan Li

Abstract

The in vitro effect of dexamethasone on the clonal growth of haematopoietic progenitors in preterm infants was investigated. Concentrations of 10^6 M to 10^9 M were associated with a dose dependent inhibition of colony formation, with the most clinically important effects seen on the earliest erythroid and granulocyte-macrophage colonies. Because of the potential clinical implications of these observations, studies are needed to determine the effects of dexamethasone on haematopoiesis in preterm infants.

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Keywords: erythroid and granulocyte-macrophage colony formation; dexamethasone phosphate; erythropoietin

Dexamethasone phosphate (DP) is a synthetic corticosteroid that is commonly administered to premature infants for the prevention or treatment of bronchopulmonary dysplasia (BPD). Its effects are mediated through specific glucocorticoid receptors.¹ In humans, glucocorticoid receptors have been shown in many different cells, including those of the granulocyte-macrophage lineage and lymphocytes.² Erythropoietic cells also have binding sites for glucocorticoids.^{3,4} DP could therefore potentially influence haematopoiesis.

As a first step toward the goal of defining the effects of DP treatment on haematopoiesis of preterm infants, we designed this in vitro study to determine whether DP affects the clonal growth of erythroid or granulocyte-macrophage progenitor cells in preterm infants, and if the preterm infant's progenitors respond to DP in a different manner from those of adults.

Methods

Blood was obtained from the umbilical vein after the placental delivery of five preterm pregnancies (25–30 weeks). Bone marrow was aspirated from the superior iliac crest of two healthy adult female volunteers. This study was approved by the University of Florida Institutional Review Board.

The effects of DP on the clonal growth of erythroid and granulocyte-macrophage progenitors were investigated using clonogenic assays according to procedures previously tested in our laboratory.⁵ In this study we started with a highly enriched population of CD34+ progenitor cells⁶ obtained by incubating the mononuclear fraction of blood and bone marrow with biotinylated anti-CD34

antibodies and running the CD34 labelled cells over an avidin column (Cellpro, Inc., Bothell, Washington, USA).

DP (Elkins-Sinn, Inc. Cherry Hill, NJ) was used at concentrations of 10^{-6} M, 10^{-7} M, 10^{-8} M, and 10^{-9} M. In the control plates no DP was added, but preservatives at a concentration equivalent to that present in 10^{-6} M DP.

For erythroid colony forming unit (CFU-E) quantification, we used 2000 cells/ml, while for erythroid burst forming units (BFU-E), colony forming units mix (CFU-mix), and granulocyte-macrophage colony-forming unit's (CFU-GM), we used 7000 cells/ml. The concentrations of erythropoietin (Epo) used to promote the growth of erythroid colonies ranged from 0.2 U/ml to 20 U/ml, based on the possibility that very high Epo concentrations might mask the effects of DP on progenitors of preterm infants. For the studies on adults, 2 U/ml Epo was used.

After colony enumeration, BFU-E and CFU-mix plates were washed and total cells counted using a cell analyser (Coulter Electronics, Inc., Chicago, IL). Differential cell counts were then performed and the absolute number of normoblasts per plate calculated.

Comparisons (Student's *t* test) were made between control (no DP added) plates and those with DP added. A *p* value of < 0.05 was considered significant.

Results

In this study DP inhibited, in a dose dependent manner, the numbers of both immature and more mature erythroid colonies (figs 1-3). Similarly, DP decreased the numbers of CFU-GM colonies ($53\pm3\%$, $55\pm8\%$, $77\pm4\%$, and $81\pm5\%$ of the baseline, from the lowest to the highest DP concentration; $p<0.05$). The number of normoblasts per erythroid colony was also reduced by the addition of DP in a dose dependent manner (table 1).

CD34+ cells from adult marrow seemed to resist the inhibitory effect of DP, and no significant differences were observed in the number of CFU-E, BFU-E, CFU-mix, and CFU-GM colonies at any DP concentration. At concentrations ranging from 0 to 10^{-6} M DP, CFU-E colonies per plate were 144 ± 16 , 127 ± 12 , 130 ± 10 , 131 ± 3 , and 124 ± 16 . At these concentrations BFU-E colonies were 14 ± 6 , 11 ± 6 , 17 ± 4 , 14 ± 4 , and 20 ± 3 per plate; CFU-MIX and CFU-GM colonies were 2 ± 1 , 5 ± 3 , 7 ± 4 , 3 ± 1 , and 14 ± 4 , and 115 ± 12 , 94 ± 7 , 91 ± 14 , 87 ± 15 , and 89 ± 9 , respectively.

Discussion

We tested the effects of DP on erythroid and granulocyte-macrophage colony formation in

Department of
Paediatrics, Division
of Neonatology,
University of Florida
College of Medicine,
P O Box 100296
Gainesville, FL, USA
P Papoff
R D Christensen
J Harcum
Y Li

Correspondence to:
Dr Yai Li.

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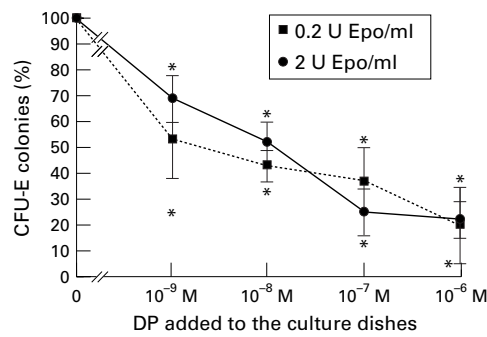


Figure 1 DP dose response curves of CFU-E colonies derived from haematopoietic progenitors in five preterm infants, stimulated with different concentrations of Epo. The number of colonies is expressed as a percentage of colonies (100%) obtained without DP. Values shown are mean (SEM) **p* < 0.05 vs control.

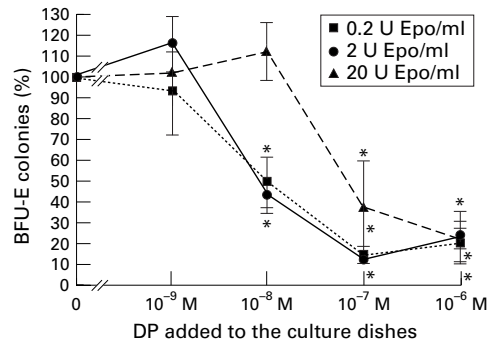


Figure 2 DP dose response curves of BFU-E colonies derived from haematopoietic progenitors in five preterm infants, stimulated with different concentrations of Epo. The number of colonies is expressed as a percentage of colonies (100%) obtained without DP. Values shown are mean (SEM) **p* < 0.05 vs control.

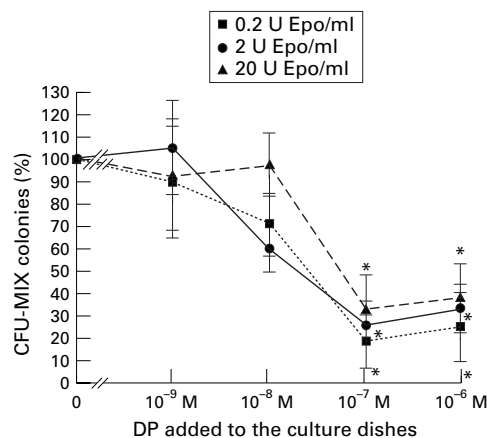


Figure 3 DP dose response curves of CFU-mix colonies derived from haematopoietic progenitors in five preterm infants, stimulated with different concentrations of Epo. The number of colonies is expressed as a percentage of colonies (100%) obtained without DP. Values shown are mean (SEM) **p* < 0.05 vs control.

progenitor cells from preterm infants and adults. The concentrations of DP included in the culture dishes ranged from 10^{-6} M to 10^{-9} M. Studies on the pharmacokinetics of DP in preterm infants have shown that the plasma concentrations reached following DP administrations are in the order of magnitude of 10^{-6} M to 10^{-8} M.⁷ It is not known, however, whether these concentrations will be achieved in the bone marrow, nor whether interactions with other hormones or mediators in the bone milieu will influence the effect of DP on haematopoietic progenitors. In our experimental conditions DP showed a dose dependent inhibitory effect on both erythroid and granulocyte-macrophage colonies derived from progenitor cells of preterm infants. CFU-E colonies were the most affected, with significant reductions observed at all concentrations of DP. This profound reduction in erythroid colonies was not apparent with bone marrow progenitors from adult individuals. This observation is consistent with previous studies that showed no significant differences in the numbers of CFU-E and BFU-E derived colonies from adult bone marrow when dexamethasone was added to the culture medium.⁸

The molecular mechanism(s) underlying the antiproliferative effects of glucocorticoids on circulating erythroid progenitors cells are not known. Glucocorticoids exert their effects by binding to a single cytoplasmatic glucocorticoid receptor. The interaction of the complex glucocorticoid-glucocorticoid receptor with steroid responsive genes produces a change in the rate of transcription, resulting in either induction or repression of the gene.¹ Therefore, one mechanism that could account for the effects of DP might involve either the induction of a protein that inhibits the proliferation of fetal erythroid progenitor cells or the repression of the genes for the receptors for colony stimulating factors.

DP also inhibited granulocyte-macrophage colonies in a dose dependent manner. In vivo, glucocorticoid treatment results in increased concentrations of circulating neutrophils.^{9 10} In experimental studies on adult bone marrow cells from mice and humans, however, the number of granulocyte-macrophage colonies seems to be consistently reduced by dexamethasone.^{11 12} Therefore, glucocorticoid may have a variable effect on each single subclass of cells deriving from granulocyte-macrophage colonies.

In conclusion, our studies show that DP inhibits erythroid and granulocyte-macrophage colony formation from progenitors of

Table 1 Effect of dexamethasone on absolute number of normoblasts that developed per plate (means (SEM))

	Normoblasts per plate				
	Control	10 ⁻⁹ M DP	10 ⁻⁸ M DP	10 ⁻⁷ M DP	10 ⁻⁶ M DP
0.2 U/ml	4188578 (1192983)	4036535 (1057815)	3734835 (650008)	2118337 (680662)	779261 (229384*)
2 U/ml	6235919 (1613897)	8022497 (2956960)	4751047 (1349938)	3057200 (1245122)	1482535 (438698*)
20 U/ml	6706827 (1873136)	5585147 (314335)	6386290 (1627679)	3717635 (287624)	1693370 (817661*)

CD34+ progenitor cells were obtained from cord blood of five preterm infants and plated in quadruplicate in culture dishes in the presence of various concentrations of Epo and DP. After 14 days, cells were washed from culture dishes, and the absolute number of normoblasts per plate was calculated. Values are mean±SEM. * *P* < 0.005 vs control.

preterm infants, in a dose dependent manner. It is not known, however, whether a clinically significant reduction in erythropoiesis and granulo-monocytopenia results from in vivo administration of DP to preterm infants. Nevertheless, in view of the potential clinical relevance, studies are needed to establish the effect of DP on haematopoiesis in preterm infants.

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