Desferoxamine preconditioning protects against cerebral ischemia in rats by inducing expressions of hypoxia inducible factor 1 α and erythropoietin

Yun-Xia LI^{1,3}, Su-Ju DING¹, Lin XIAO², Wei GUO², Qing ZHAN³

¹The Changhai Hospital Affiliated to the Second Military Medical University, Shanghai 200433, China

²Neurology Institute, the Second Military Medical University, Shanghai 200433, China

³Department of Neurology, Tongji Hospital Affiliated to Tongji University, Shanghai 200065, China

Abstract: Objective To investigate whether desferoxamine (DFO) preconditioning can induce tolerance against cerebral ischemia and its effect on the expression of hypoxia inducible factor 1 α (HIF-1 α) and erythropoietin (EPO) *in vivo* and *in vitro*. **Methods** Rat model of cerebral ischemia was established by middle cerebral artery occlusion with or without DFO administration. Infarct size was examined by TTC staining, and the neurological severity score was evaluated according to published method. Cortical neurons were cultured under ischemia stress which was mimicked by oxygen-glucose deprivation (OGD), and the neuron damage was assessed by MTT assay. Immunofluorescent staining was employed to detect the expressions of HIF-1 α and EPO. **Results** The protective effect induced by DFO (decreasing the infarction volume and ameliorating the neurological function) appeared at 2 d after administration of DFO (post-DFO), lasted until 7 d and disappeared at 14 d ($P < 0.05$); the most effective action was observed at 3 d post-DFO. DFO induced tolerance of cultured neurons against OGD: neuronal viability was increased 23%, 34%, 40%, 48% and 56% at 8 h, 12 h, 24 h, 36 h, and 48 h, respectively, post-DFO ($P < 0.05$). Immunofluorescent staining found that HIF-1 α and EPO were upregulated in the neurons of rat brain at 3 d and 7 d post-DFO; increase of HIF-1 α and EPO appeared in cultured cortex neurons at 36 h and 48 h post-DFO. **Conclusion** DFO induced tolerance against focal cerebral ischemia in rats, and exerted protective effect on OGD cultured cortical neurons. DFO significant induced the expression of HIF-1 α and EPO both *in vivo* and *in vitro*. DFO preconditioning can protect against cerebral ischemia, which may be associated with the synthesis of HIF-1 α and EPO.

Keywords: desferoxamine; ischemia preconditioning; hypoxia inducible factor 1 α ; erythropoietin

1 Introduction

There are a number of clinical conditions under which cerebral ischemia can be anticipated (e.g. surgery of the heart or brain, subarachnoid hemorrhage). It is desirable to develop clinically applicable approaches to induce ischemia-tolerant state of the brain. However, few published experimental protocols for ischemic preconditioning can be applied to humans.

Desferoxamine (DFO) is an iron chelator which has been licensed to treat hemochromatosis caused by iron overload from repeated blood transfusion in anemia patients. It can increase hematocrit through upregulation of hypoxia-inducible transcription factor 1 (HIF-1) and the major stimulator of erythropoiesis, erythropoietin (EPO). HIF-1 is a master switch of the brain response to hypoxia, and EPO is a potent neuroprotectant *in vivo* and *in vitro*^[1].

In this study, we investigate whether precondition of DFO can protect the brain against ischemia insult by inducing the synthesis of HIF-1 α and EPO. In addition, we explored the effect of DFO on neurons in culture to eliminate the possibility that DFO induces changes in systemic circulatory parameters, vascular reactivity, or glia-neuron interactions.

Corresponding author: Su-Ju DING
Tel: 86-21-25070615
E-mail: dingsuju@hotmail.com
Article ID: 1673-7067(2008)02-0089-07
CLC number: R743
Document code: A
Received date: 2007-12-27

2 Materials and methods

2.1 Materials DFO, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma, St. Louis, MO, USA); 2,3,4-triphenyltetrazolium chloride (TTC; Shanghai Jinkuang Co., China); mouse anti-microtubule associated protein 2 (MAP2; a neuron specific marker) monoclonal antibody, rabbit polyclonal anti-HIF1 α antibody, rabbit polyclonal anti-EPO antibody, FITC conjugated goat anti-rabbit IgG antibody, CY3 conjugated goat anti-mouse IgG antibody (Wuhan boshide Co., China); poly-L-lysine, Trypsin, horse serum, fetal bovine serum, Dulbecco's modified Eagle's medium (DMEM), Neurobasal medium, and B27 (Gibco BRL, Grand Island, USA). All other used chemicals were analytical grade.

2.2 Assessment of infarct size and neurological severity score (NSS) All treatments for animals were approved by the Institutional Animal Care and Use Committee of the Second Military Medical University, China. Procedures were designed to minimize the number of animals used in experiments and to reduce their suffering. Eighteen male Sprague-Dawley rats (280-320 g) were randomly divided into 3 groups (6 rats in each group): DFO group, normal saline (NS) group and sham-operation group. DFO (100 mg/kg, i.p.) was administered to rats in the DFO group, while an equivalent volume of saline was administered intraperitoneally to the NS group and the sham-operation group. At 1, 2, 3, 7, or 14 d after administration of DFO (post-DFO), the middle cerebral artery suture occlusion (MCAO) was performed in the rats of DFO group and NS group to produce transient (2 h) focal cerebral ischemia, as described by Longa EZ *et al.*^[2]. Animals were anesthetized with 40 mg/kg pentobarbital. Rectal temperature was monitored and maintained at 36 °C-37 °C by combined use of overhead lights and heating blanket. Only the rats which survived until defined time and exhibited Homer symptoms in the ipsilateral eye and palsy in the contralateral anterior limb after they recovered consciousness were identified as the successful model.

Neurological function was assessed at 24 h post-MCAO by neurological severity scores (NSS)^[3]. NSS is a composite of motor, sensory, reflex and balance tests. Neurological function was graded on a scale of 0 to 18 (normal score, 0; maximal deficit score, 18; Tab. 1); and the higher score, the more severe is the injury.

The infarct size of rat brain was determined by TTC

staining. Rats were decapitated after MCAO. The brains were removed quickly and sectioned coronally into six slices (2 mm for each), beginning from the frontal pole of the cortex. The slices were incubated in 2% TTC at 37.8 °C and fixed in 10% buffered formalin. The unstained areas of fixed brain slices were defined as the infarcted. Infarct size was determined by using a charge-coupled device (CCD) camera and image analysis software Image Tool 3.0 as Gerriets T *et al.* described^[4].

2.3 Preparation of cortex neurons The cerebral cortex cells were obtained from fetal Sprague-Dawley rats [embryonic day 18 (E18)] as previously reported^[5]. Cortex was dissected in ice-cold dissection solution containing sucrose, glucose and HEPES. The isolated cortex was mechanically triturated, and then digested in solution containing 0.25% trypsin and 1 mmol/L EDTA at 37 °C for 15 min. Single cell suspension was obtained by repeatedly passaging dissociated tissues by fire-polished pipette in the DMEM which was supplemented with 10% heat-inactivated fetal bovine serum and horse serum. Cells at optimal densities were finally plated on the 0.1 mg/mL poly-L-lysine coated plates, dishes, or glass coverslips in different experiments. Within 24 h after plating, the serum-containing plating medium was replaced by a kind of serum-free Neurobasal medium which was supplemented with 2% B27 (culture medium). Half of the culture medium was changed every 3 d thereafter. More than 95% cells were verified as neurons by MAP2 staining on day 7 *in vitro* (7DIV). All experiments were carried out on the 7DIV unless indicated otherwise.

2.4 Neuronal viability and cytotoxicity assay The viability of neurons was assessed by MTT assay. Briefly, cortex neurons in 96-well plates (about 40 000 cells per well) were pre-treated with 300 μ mol/L of DFO for 24 h in the plating medium under normoxic, humidified conditions, and then cultured in normal medium. A positive control was set by adding DFO (300 μ mol/L) to the culture. At different time points post-DFO (2, 6, 8, 12, 24, 36 or 48 h), the neurons underwent 120-min oxygen and glucose deprivation (OGD) by replacing the culture medium with an anoxic and glycoprivial solution in an anaerobic chamber ($P_{O_2} < 1$ mmHg). After OGD, the remaining medium in the wells were replaced by 100 μ mol/L fresh MTT solution dissolved in DMEM (0.5 mg/mL). After incubation at 37 °C for another 3 h, the supernatant were removed and the formazan crystals were dissolved in 100 μ L acidic

Tab. 1 Neurological Severity Scores (NSS)

	Points
Motor tests	
Raising rat by the tail	3
1 Flexion of forelimb	
1 Flexion of hindlimb	
1 Head moved >10° to vertical axis within 30 s	
Placing rat on the floor (normal = 0; maximum = 3)	3
0 Normal walk	
1 Inability to walk straight	
2 Circling toward the paretic side	
3 Fall down to the paretic side	
Sensory tests	
1 Placing test (visual and tactile test)	2
2 Proprioceptive test (deep sensation, pushing the paw against the table edge to stimulate limb muscles)	
Beam balance tests	6
0 Balances with steady posture	
1 Grasps side of beam	
2 Hugs the beam and one limb falls down from the beam	
3 Hugs the beam and two limbs fall down from the beam, or spins on beam (> 60 s)	
4 Attempts to balance on the beam but falls off (> 40 s)	
5 Attempts to balance on the beam but falls off (> 20 s)	
6 Falls off: No attempt to balance or hang on to the beam (> 20 s)	
Reflexes absent and abnormal movements	4
1 Pinna reflex (head shake when touching the auditory meatus)	
1 Corneal reflex (eye blink when lightly touching the cornea with cotton)	
1 Startle reflex (motor response to a brief noise from snapping a clipboard paper)	
1 Seizures, myoclonus, myodystony	
Maximum points	18

isopropanol (0.04 mol/L HCl in isopropanol). The viability of neuron was measured by the absorbance at 570 nm. The ratio of neuron survival was calculated by normalizing to positive control. Neurons were viewed and photographed under an Olympus microscope before the assays.

2.5 Indirect double immunofluorescence staining Twelve male Sprague-Dawley rats (280-320 g) were randomly divided into DFO group and NS group (6 rats in each group): intra-peritoneally administered with 100 mg/kg DFO and equivalent volume of saline, respectively. The brains were removed 1, 2, 3, 7, or 14 d later and fixed in 10% formalin at 4 °C for 8-12 h. After being stored in 30% sucrose in 0.1 mol/L PB at 4 °C overnight for dehydration, the brains were embedded in Paraplast (Sigma, St. Louis, MO, USA) and cut at 15 μ m

coronally on a cryostat microtome (Leica, Germany). The frozen sections were collected in 0.01 mol/L PBS containing 0.1% sodium azide, following permeabilization with 0.3% Triton X-100 in PBS for 5 min. Rabbit anti-HIF-1 α , anti-EPO and mouse anti-MAP2 (all 1:100 in diluted in PBS containing 5% normal goat serum) were incubated with the slices overnight at 4 °C to detect the expression of HIF-1 α , EPO and MAP2, respectively. The final detection was made by incubation with FITC-conjugated anti-rabbit IgG antibody and CY3-conjugated anti-mouse IgG antibody (1:200 diluted in PBS containing 5% normal goat serum) for 1 h at 37 °C. The Olympus fluorescence microscope (IX70) which was connected to a personal computer via a CCD camera was used to visualize the slices. The photographs were analyzed by

MetaMorph image analysis software.

The cultured cortex neurons grown on glass coverslips were divided into 2 groups: DFO group, neurons were pretreated with 300 $\mu\text{mol/L}$ DFO for 24 h in the plating medium, and then cultured in normal medium; and control group, neurons were normally cultured without DFO pretreatment. Forty eight hours later after DFO pretreatment, the coverslips were washed 3 times with PBS for 5 min each, and cells were fixed with 4% paraformaldehyde for 20 min at room temperature. The following procedure went as mentioned above of the brain sections.

2.6 Statistical analysis The statistical significance was assessed by one way analysis of variance (ANOVA) with Student's *t*-test. $P < 0.05$ was considered statistically significant.

3 Results

3.1 Assessment of NSS and infarct size In the model of focal cerebral ischemia, intraperitoneal injection of 100 mg/kg DFO significantly protected the brain against the 120-min MCAO. NSS in the DFO group was 7.91 ± 0.89 (1 d), 7.26 ± 1.09 (2 d), 5.6 ± 0.54 (3 d), 6.97 ± 0.89 (7 d), and 7.89 ± 1.14 (14 d); the neurological function ameliorated at 2, 3 and 7 d post-DFO compared with the NS group (8.02 ± 1.14) ($P < 0.05$, Fig. 1A).

The percentage of brain infarct area was $(45.5 \pm 3.4)\%$ (1 d), $(44.5 \pm 2.4)\%$ (2 d), $(31.3 \pm 2.5)\%$ (3 d), $(41.3 \pm 3.3)\%$ (7 d), and $(46.43 \pm 1.9)\%$ (14 d); decreased 12%, 32.3% and 10.6%, respectively, at 2, 3 and 7 d post-DFO as compared with the NS group ($P < 0.05$, Fig. 1B, C).

The protective effect of DFO (decreasing the infarction

Fig.1 Time kinetics of desferrioxamine (DFO, 100 mg/kg body weight) induced ischemia tolerance in rats. A: Neurological severity scores; B: percentage of infarct size. Sham: sham operation group; NS: normal saline group. The protective effect appeared at 2 d post-DFO, lasted until 7 d, and disappeared at 14 d. The most effective action was observed at 3 d post-DFO. $*P < 0.05$ vs NS; C: TTC staining of the brain; the white part was defined as infarct area. Scale bar, 5 mm.

Fig. 2 Desferrioxamine (DFO, 300 $\mu\text{mol/L}$) induces tolerance of primary cortical neurons against oxygen-glucose deprivation (OGD) in rats. The neuronal viability increased gradually 8 d later after DFO pretreatment. Normal: under nonhypoxia condition; OGD: no DFO pretreatment. $*P < 0.05$ vs OGD.

volume and ameliorating the neurological function) appeared at 2 d post-DFO, lasted until 7 d, and disappeared at 14 d; The most effective action was observed at 3 d post-DFO.

3.2 Neuronal viability and cytotoxicity assay Similar to the *in vivo* condition, DFO time-dependently induced tolerance of cultured neurons against OGD. The neuronal viability was $(35 \pm 1)\%$ (2 h), $(34 \pm 2)\%$ (6 h), $(55 \pm 2)\%$ (8 h), $(68 \pm 3)\%$ (12 h), $(72 \pm 2)\%$ (24 h), $(80 \pm 3)\%$ (36 h), and $(86 \pm 2)\%$ (48 h) (Fig. 2). The protective effect appeared at 8 h post-DFO, when neuronal viability increased 23% compared with OGD group [$(32 \pm 3)\%$]. The protective effect was stronger with the time prolongation:

Fig. 3 Expression of HIF-1 α and EPO in brain tissue of rats. NS pretreatment was set as control. HIF-1 α and EPO positive staining were enhanced at 3 d after DFO pretreatment. Scale bar, 50 μ m.

Fig. 4 Expression of HIF-1 α and EPO in the cultured neurons. NS pretreatment was set as control. HIF-1 α and EPO positive staining were observed at 48 h after DFO pretreatment. Scale bar, 25 μ m.

neuronal viability increased 34% at 12 h post-DFO; increased 40% at 24 h; increased 48% at 36 h, and the maximum protection (increased 56%) was obtained at 48 h post-DFO ($P < 0.05$). The 72-h interval was not tested for the limited time of purified primary cortical neurons kept in cultures.

3.3 Indirect double immunofluorescence staining The synthesis of HIF-1 α and EPO induced by DFO were observed in rat brains and cultured cortex neurons. In the neuronal cells of rat brains, HIF-1 α and EPO positive staining were enhanced at 3 d (Fig. 3) and 7 d (data not shown) post-DFO, peaked at 3 d, and disappeared at 14 d. In the cultured cortex neurons, HIF-1 α and EPO positive staining were observed at 36 h (data not shown) and 48 h post-DFO (Fig. 4). The 72-h interval was not tested.

4 Discussion

We found that DFO induced tolerance of brain against reversible focal cerebral ischemia *in vivo* and *in vitro*; the protective effect (decreasing the infarction volume and ameliorating the neurological function) appeared at 2 d post-

DFO, the most effective action was observed at 3 d and lasted until 7 d, and disappeared at 14 d. In cultured neurons, the effective action was observed at 8 h post-DFO, the maximum protection was at 48 h and the longer effect could not be observed. Thus the *in vitro* effects of DFO were in agreement with the *in vivo* effects, indicating that DFO-induced ischemic tolerance might be explained, at least in part, by the effect of neurons in the brain. The fluorescent staining revealed that DFO induced the expression of HIF-1 α and EPO at 3 d and 7 d post-DFO in brain tissues, and at 36 h and 48 h post-DFO in cultured neurons, which were coincided with the time of DFO functioning.

Chavez *et al.* found that HIF-1 α was closely related with cerebral ischemia, and played an important role in protection of brain against cerebral ischemia^[6]. The decrease of cerebral blood flow may reduce the oxygen supply of the penumbra, and thus induce the expressions of HIF-1 α and its target genes such as EPO. HIF-1 α is beneficial for cell survival in ischemia tissues, and it might be involved in the protection mechanism of ischemic preconditioning^[7].

Many studies, including cell culture and animal experiments, have proved that EPO is expressed in the nervous system during ischemia-hypoxia^[8,9]. Our data are in agreement with the recent literature that the ischemia tolerance of brain is associated with HIF-1 α and EPO. We found that the tolerance induced by DFO was time-dependent, i.e. the maximum effect appeared at 3 d and 48 h post-DFO in MCAO rats and OGD neurons, respectively, which is coincided with the expression time of HIF-1 α and EPO. However, the correlation among these changes was not proved by the present study, nor could which rule out the possible effect of other gene products. HIF-1 α is involved in regulation of glucose transporters and glycolytic enzymes, and basic cell proliferation and survival^[10]. Of particular relevance is that HIF-1 α induces the expression of receptors and cytokines for growth and regulates functions of vasculature, which was one of the reasons why we sought to confirm our *in vivo* data in the absence of vasculature and blood flow.

However, one has to consider other possible pathways by which DFO may promote cell survival, e.g. blocking the formation of reactive oxygen species as an iron chelator. Furthermore, DFO can block the cell cycle, and may thereby prevent neuronal cells from death^[11]. Recently, a number of investigators have used various *in vivo* and *in vitro* models to show that EPO and its receptor were expressed in the brain and acted as endogenous neuroprotectants, and the exogenous delivery of EPO is a promising strategy for the management of stroke^[12]. The mechanism of EPO's neuroprotective effect is currently under intense investigation, but a common conclusion seems to be its transcriptional or posttranslational anti-apoptotic effect.

In humans, DFO is given in doses of up to several grams per day. In our experiments, DFO was given at the dose of 100 mg/kg body weight, approximately 10-fold higher than that for humans. Because of the higher metabolism and excretion in rats, it is not uncommon that equivalent doses (and unwanted side effects) for rats are at least 10 times higher than that for humans.

Since DFO is safe enough to not affect systemic hematocrit after a single dose, it may be an interesting candidate for induction of an ischemia tolerant state of the brain in humans.

Acknowledgements: This work was supported by the

National Natural Science Foundation of China (No. 30170340).

References:

- [1] Prass K, Ruscher K, Karsch M, Isaev N, Megow D, Priller J, *et al.* Desferrioxamine induces delayed tolerance against cerebral ischemia *in vivo* and *in vitro*. *J Cereb Blood Flow Metab* 2002, 22: 520-525.
- [2] Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke* 1989, 20: 84-91.
- [3] Chen J, Li Y, Wang L, Zhang Z, Lu D, Lu M, *et al.* Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke* 2001, 32: 1005-1011.
- [4] Gerriets T, Li F, Silva MD, Meng X, Brevard M, Sotak CH, *et al.* The macrosphere model: evaluation of a new stroke model for Permanent middle cerebral artery occlusion in rats. *J Neurosci Methods* 2003, 122: 201-211.
- [5] Murphy TH, Miyamoto M, Sastre A, Schnaar RL, Coyle JT. Glutamate toxicity in a neuronal cell line involves inhibition of cystine transport leading to oxidative stress. *Neuron* 1989, 2: 1547-1558.
- [6] Chavez JC, Agani F, Pichiule P, LaManna JC. Expression of hypoxia-inducible factor-1 alpha in the brain of rats during chronic hypoxia. *J Appl Physiol* 2000, 89: 1937-1942.
- [7] Tan XJ, Hu CL. Therapeutic effect of hypoxia-inducible factor-1 α on focal cerebral ischemia: experiment with rats. *Nat Med J Chin* 2006, 86: 1057-1060. (Chinese, English abstract)
- [8] Sun Y, Zhou C, Polk P, Nanda A, Zhang JH. Mechanisms of erythropoietin induced brain protection in neonatal hypoxia ischemia rat model. *J Cereb Blood Flow Metab* 2004, 24: 259-270.
- [9] Liu J, Narasimhan P, Yu F, Chan PH. Neuroprotection by hypoxic preconditioning involves oxidative stress-mediated expression of hypoxia-inducible factor and erythropoietin. *Stroke* 2005, 36: 1264-1269.
- [10] Freret T, Valable S, Chazalviel L, Saulnier R, Mackenzie ET, Petit E, *et al.* Delayed administration of deferoxamine reduces brain damage and promotes functional recovery after transient focal cerebral ischemia in the rat. *Eur J Neurosci* 2006, 23: 1757-1765.
- [11] Katchanov J, Harms C, Gertz K, Hauck L, Waeber C, Hirt L, *et al.* Mild cerebral ischemia induces loss of cyclin-dependent kinase inhibitors and activation of cell cycle machinery before delayed neuronal cell death. *J Neurosci* 2001, 21: 5045-5053.
- [12] Li Y, Lu Z, Keogh CL, Wei L. Erythropoietin-induced neurovascular protection, angiogenesis, and cerebral blood flow restoration after focal ischemia in mice. *J Cereb Blood Flow Metab* 2007, 27: 1043-1054.

去铁敏预处理通过增加缺氧诱导因子 1 α 和促红细胞生成素的表达减轻大鼠缺血性脑损伤

李云霞^{1,3}, 丁素菊¹, 肖林², 郭卫², 詹青³

¹第二军医大学附属长海医院神经内科, 上海 200433

²第二军医大学神经生物研究所, 上海 200433

³同济大学附属同济医院神经内科, 上海 200065

摘要: 目的 观察去铁敏 (Desferoxamine, DFO) 预处理后大鼠脑组织和体外培养神经元中缺氧诱导因子 1 α (hypoxia inducible factor 1 α , HIF-1 α) 和促红细胞生成素 (erythropoietin, EPO) 表达的变化, 探讨预处理是否对体内及体外的脑缺血损伤的具有保护效应。**方法** 去铁敏预处理大鼠后不同时间点制作大脑中动脉阻塞 (middle cerebral artery occlusion, MCAO) 模型, 术后 24 h 后处死动物。采用神经功能评分 (neurological severity scores, NSS) 和计算梗死体积 (TTC 染色) 评价 DFO 的脑保护效应, 细胞活力测定评价 DFO 对缺氧缺糖条件下 (oxygen-glucose deprivation, OGD) 皮层神经元的保护效应。免疫荧光染色检测 HIF-1 α 和 EPO 蛋白表达情况。**结果** 与生理盐水对照组比较, 去铁敏预处理后 2 d, MCAO 大鼠出现梗塞面积缩小, 神经功能损伤减轻, 在预处理后 3 d 达到高峰, 7 d 仍然有效, 14 d 去铁敏预处理的保护效应消失。去铁敏对 OGD 神经元同样具有神经保护作用: 与未进行预处理的神经元细胞相比, 预处理后 8 h 的细胞活力增加 23%, 12 h 增加 34%, 24 h 增加 40%, 36 h 增加 48%, 48 h 增加 56% ($P < 0.05$)。免疫荧光染色发现, 大鼠脑组织的 HIF-1 α 和 EPO 在去铁敏预处理后 3 d 及 7 d 表达上调; 皮层神经元细胞的 HIF-1 α 和 EPO 在去铁敏预处理后 36 h 及 48 h 表达上调。**结论** 去铁敏预处理有确切有效的脑保护效应, 不仅可以预防脑缺血损伤, 对体外培养的 OGD 皮层神经元细胞损伤也具有保护作用, 其机制可能与脑神经细胞的 HIF-1 α 和 EPO 蛋白表达增加有关。

关键词: 去铁敏; 缺血预适应; 缺氧诱导因子 1 α ; 促红细胞生成素