Study on the Trophic Effect of Human Recombinant Erythropoietin on the Developing Small Bowel in Neonatal Rats

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ABSTRACT

The present work was done to study the trophic effect of human recombinant erythropoietin on the developing small bowel in neonatal rats. This study was carried out on 24 neonatal rats aged 4-5 days, weighing 40-60gm, and divided into 4 groups each containing 6 neonatal rats: Groups (1): was the control, group (2): administrated enteral human recombinant erythropoietin(Epo) through intragastric tube in a dose of 200 u/kg/day for one week, group (3): administrated enteral human recombinant Epo in a dose of 1000 u/kg/day through intragastric tube for one week, and group (4): administrated human recombinant Epo parenterally in a dose of 200u/kg/day for one week. At the end of the experiment, neonatal rats were weighed and blood was collected by cardiac puncture, sacrificed , dissected and small intestine was excised, length was measured and fixed in paraffin for histological examination. The results showed that administration of enteral recombinant Epo in dose of 200 and 1000 u/kg/day for a week caused significant increase in body weight and small bowel length , and non significant effect on haematocrit value or plasma erythropoietin concentration. The parenteral administration of Epo showed, significant increase in body weight, haematocrit value, plasma erythropoietin concentration and small bowel length. Histological examination showed, increased surface area of intestinal mucosa and increased length of the ilial villi.. It is concluded that, parenteral administration of human recombinant Epo has haematopoietic and trophic effects on the small bowel. Enteral administration of human recombinant Epo has a local trophic effect on small bowel, which is useful in treatment of infants suffering from defective absorption due to short bowel syndrome.

INTRODUCTION

Erythropoietin (Epo) is a glycoprotein with a true hormonal structure which is located in the alpha-globulin of the plasma[(1)]. It is one of the growth factors that have a positive effect on haematopoietic growth and wound healing[(2)]. Epo is present in human milk, amniotic fluid[(3)], bone marrow and spleen [(4)]. Epo receptors are present in the intestinal villi of developing humans and rats[(5)]. These Epo receptors appear to be functional, as recombinant Epo increases the cell migration and decreases apoptotic death following damage and has trophic effects on cultured gastric mucosal cells[(6)]. The Epo receptors are present on enterocytes, and they are a

readily accessible source of its ligand which is available to the fetus and breast fed neonates[5]. The possible physiological roles of Epo in the developing bowel are systemic erythropoietic effect and local effect[7]. Epo might have local effect on developing bowel, as rats treated with parenteral recombinant Epo prior to a surgical anastomosis showed improved healing with increased strength of the anastomosis [7]. Also, infants who received recombinant Epo for anaemia of prematurity showed, decreased incidence of necrotising enterocolitis compared to infants who did not receive recombinant Epo[9].

The aim of the present work was to study the trophic effect of human recombinant erythropoietin on the developing small bowel of neonatal rats.

MATERIALS & METHODS

24 neonatal rats of 4-5 days age, and weighing 40-60gm, were kept with their mothers in containers containing dust free maize cob bedding and at conditioned temperature at 37ºC. The rats were fed freely from their mothers, which were fed by milk and bread and had free water access with regular perfect cleaning.

The neonatal rats were divided into four groups each containing 6 rats.

Groups (1): is the control group, administrated 1/2 ml saline by small intra- gastric tube (PE-10) from (Sigma) for one week.

Groups (2): administrated enterally by human recombinant Epo (Amoun) in a dose of 200u/kg/day [7] by intragastric tube for one week.

Group (3): administrated enterally by human recombinant Epo in a dose of 1000u/kg/day by intragastric tube for one week.

Group (4): administrated parenterally by human recombinant Epo (Amoun) in a dose of 200 u/kg/day[7] intraperitonealy for one week .

At the end of the experimental period, rats were weighed, and blood was collected by cardiac puncture for estimation of:

Haematocrit value% measured by the method of Juul [7].

Erythropoietin concentration was measured by duplication with quanntikine IVP ELISA kit (R&D systems. Minneapolis) which based on the Double-Antibody Sandwich Method with the use of monoclonal antibody by the method of Jazayeri et al. [10].

Histological examination:

The animals were sacrificed, dissected. The abdomen was opened, the entire small intestine was removed and length was measured. Sections of the ilium were taken, kept in formalin and fixed in paraffin blocks for histological examination.

Statistical analysis

All results were expressed as mean values $\pm$ standard deviation (SD). Mean values of different groups were compared using one way analysis of variance. The least significant different mean values, $P< 0.05$ was accepted to denote a significant difference.

RESULTS

The results of the present work are shown in table (1) and figures(1-4):

Effects of enteral administration of human recombinant Epo to neonatal rats in a dose of 200u/kg for a week: The results showed significant increase in the body weight compared with the control, P<0.05. There was non significant change of haematocrit value %or plasma erythropoietin concentration compared with the control.

Effects of enteral administration of human recombinant Epo to neonatal rats in a dose of 1000u/kg for a week: The results showed significant increase in the body weight compared with the control, P< 0.05. Also, there was non significant change of haematocrit value % or plasma erythropoietin concentration compared with the control.

Effects of parenteral administration of human recombinant Epo in a dose of 200u/day for a week: The results showed, significant increase in the body weight, haematocrit value % and plasma erythropoietin concentration compared with the control, P< 0.05. There was significant increase of haematocrit value % compared with the enterally administrated groups by Epo in a dose of 200 or 1000 u/kg/day, P<0.05.. In addition, there was significant increase of plasma erythropoietin concentration compared with the enterally administrated groups by Epo in a dose of 200 or 1000u/kg, P< 0.05.

The results showed non significant change between enterally administrated groups by Epo in a dose of 200 or 1000u/kg in body weight, haematocrit value % and plasma erythropoietin concentration.

Effect of enteral and parenteral administration of human recombinant Epo on the small bowel length: The results showed significant increase of small bowel length after enteral and parenteral Epo in a dose of 200 or 1000u/kg compared with the control, p<0.05. There was a significant increase of small bowel length by enteral Epo in a dose of 1000u/kg compared with the parenteral Epo group, P<0.05.

Table (1): Effects of enteral and parenteral administration of human recombinant Epo on the body weight (gm), haematocrit value %, plasma erythropoietin concentration (mu/mL) and length of small bowel (cm) in neonatal rats (mean 6± SD).

Parameter	Control	Enteral 200u/kg	Enteral 1000u/kg	Parenteral 200u/day
Body weight (gm)	79±2.36	85.7±2.32*	86.6±1.5*	86±1.98#*
Haematocrit value %	41.1±2.31	41.2±2.03	42.1±2.32	42.8±2.12 # *
Plasma erythropoietin (mu/mL) concentration	14.6±0.71	14±1.12	14.4±0.81	24.9±0.99# *
Small bowel length (cm)	50.7±0.7	52.8±0.82*	53.2±1.04 # *	52.08±.34*

**= Denotes statistical significance compared with control.*
#= Denotes statistical significance between enterally and parenterally administrated groups.

Histological examination

Examination of ilium showed increased surface area of the ilial mucosa, and increased length of the ilial villi in neonatal rats administrated enteral and parenteral human recombinant Epo, (Figures 5-7).

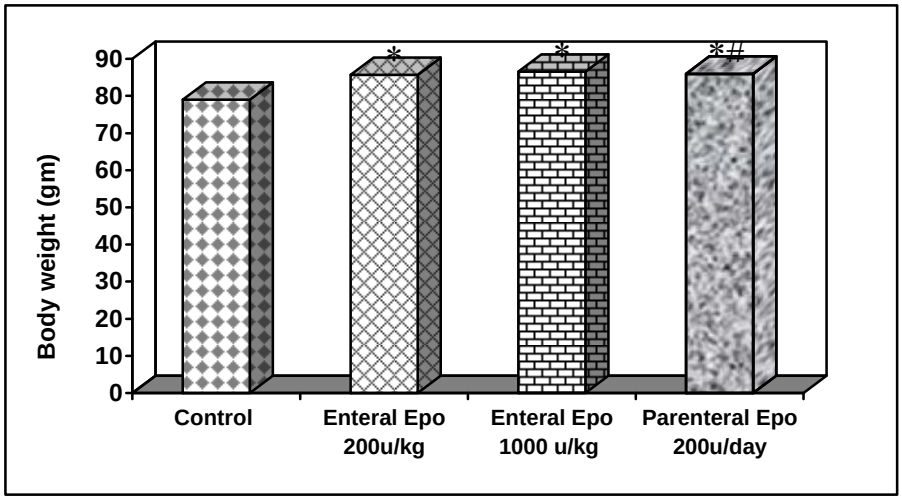


Fig (1): Effect of enteral and parenteral administration of human recombinant Epo on body weight (gm) in neonatal rats.

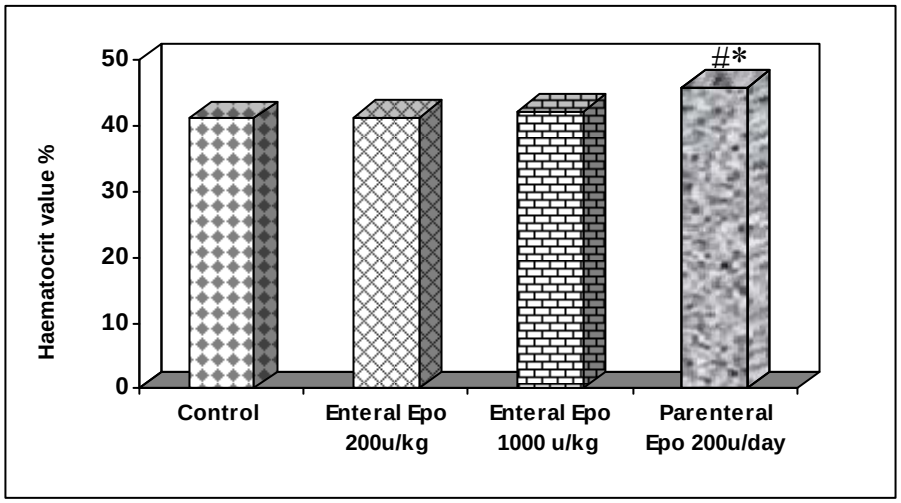


Fig (2): Effect of enteral and parenteral administration of human recombinant Epo on haematocrit value % in neonatal rats

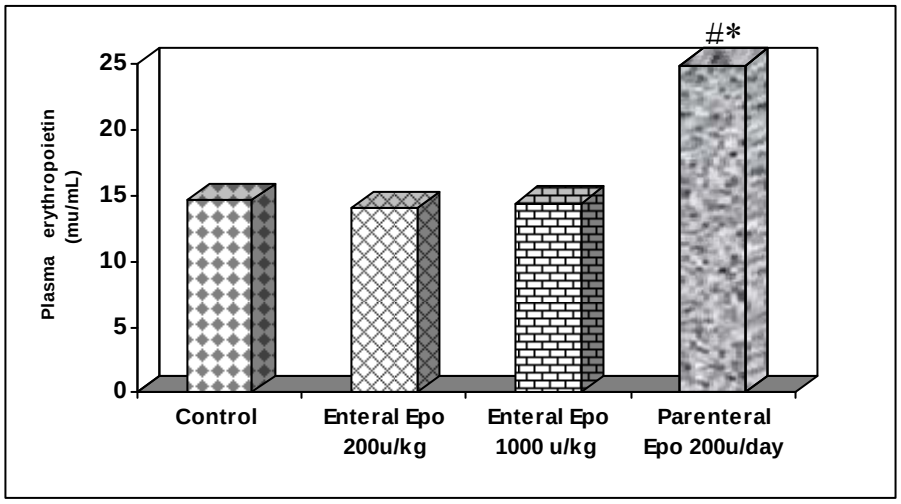


Fig (3): Effect of enteral and parenteral administration of human recombinant Epo on plasma erythropoietin concentration (mu/mL) in neonatal rats.

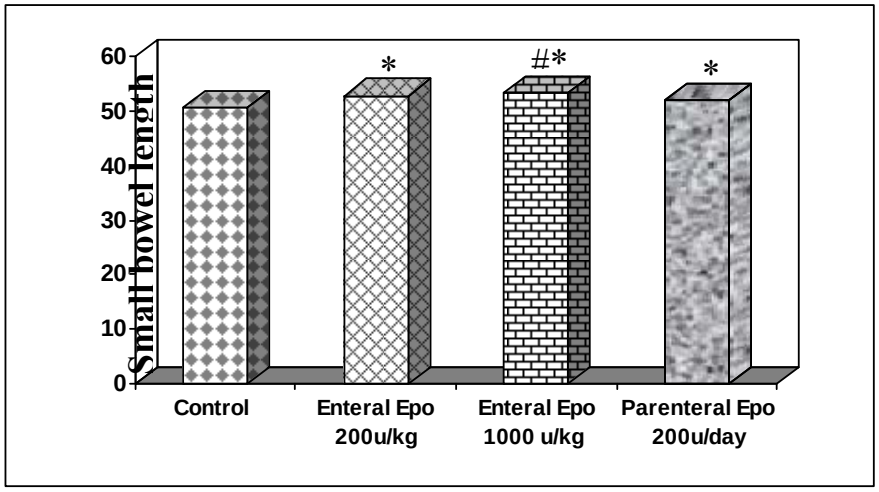


Fig (4): Effect of enteral and parenteral administration of human recombinant Epo on small bowel length (cm) in neonatal rats.

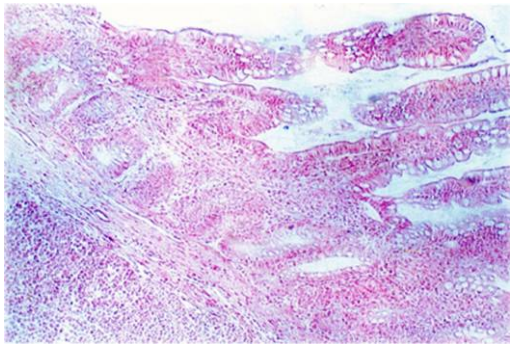


Fig (5): Section of ilium of neonatal control rat showing finger-like villi, and they are short over Payer's patches and lamina propria contains Payer's patches extend to submucosa with more numerous goblet cells(H&E 200x)

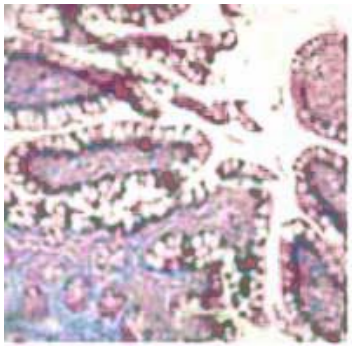
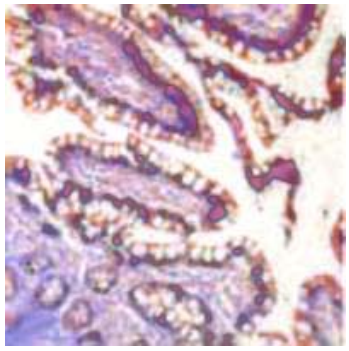


Fig (6): Section of ilium of neonatal rat enterally administrated by human recombinant Epo showing increased surface area of the ilial mucosa and increased villus length with more abundant goblet cells(H& E 200x).



Fig(7): Section of ilium of neonatal rat parenterally administrated by human recombinant Epo showing, increased surface area of the ilial mucosa and increased villus length with abundant goblet cells(H&E 200x).

DISCUSSION

Erythropoietin (Epo) is a haematopoietic growth factor with a true hormonal structure, located in the alpha globulin fraction of plasma, and has a molecular weight of 46.000 Kilo daltons[(2)] .Like growth hormone, it is also a member of the haematopoietic super family which consists of growth hormone, Epo, granulocyte and macrophage colony stimulating factors and interleukin 3,4,6,7. All of them have similarities in their receptor structure, and cross-reactivity between certain growth factors may be exist[(11)]. Epo is produced mainly in the kidneys and to a lesser extent in the liver[(12)]. Circulating Epo binds to Epo receptors on the surface of erythroid progenitors resulting in the replication and maturation to functional

erythrocytes[13]. Epo receptors have also been found in the kidneys, liver, brain, intestine, bone marrow and cardiomyocytes[14]. Also, it is possible that Epo had an anabolic effect on wound healing by affecting other growth factor receptors, thus Epo may increase fibroblast proliferation, collagen deposition, endothelial cell proliferation (angiogenesis) and the manufacture of extra cellular matrix[15]. The results of the present work showed that enteral and parenteral recombinant Epo caused increase of growth of the small bowel, including mucosal surface area and length of the villi. Also, the enterally administrated recombinant Epo had more prominent growth effect than parenteral Epo. This can be supported by the presence of Epo in the amniotic fluid and milk which have an important trophic effect in the developing bowel[16]. It may be explained by the hypothesis that, Epo can be enterally absorbed, as proved by increase erythropoiesis in nursing neonatal rats following maternal phlebotomy (to stimulate erythropoietin release), which proved that Epo may be transmitted through mothers milk in rodents[17]. The results showed non significant increase of plasma Epo concentration and haematocrit value % by enteral administration of human recombinant Epo. Parenteral administration of Epo showed, significant increase of plasma Epo concentration. This can be explained by the significant role of Epo receptors present in rat small bowel in absorption of enteric Epo. It is possible that, the use of human recombinant Epo may have decreased the enteric absorption compared to endogenous rat Epo, as rat and human Epo are only 80-82% homologous[18]. However, human and rat Epo show biological and immunological cross reactivity as demonstrated by the increase of haematocrite values in parenterally treated group[17]. Moreover, the enterally administrated recombinant Epo would have trophic effects in the small bowel, which was proved after one week treatment. Histological examination showed increase of small bowel length, mucosal surface area and length of the ilial villi[20]. This may be due to activation of Epo receptors gene expression, that is essential for activation of growth and differentiation of Epo-dependent cell lines[21], which is necessary to delay Epo expression in erythriod progenitors until Epo receptors gene activation which is needed for erythriod cell expansion [22]. Moreover it was suggested that Epo had antiapoptotic effect which may increase the process of development of bowel [14]. It is possible that, the effects of human recombinant Epo on small bowel would be route dependent. The results showed that Epo whether given enterally or parenterally, had a trophic effect on small bowel, but the enterally administrated Epo had a greater effect than parenterally Epo on small bowel length , and associated with increase in villus length and mucosal surface area, which is more pronounced in the ilium[23]. The mechanism of increase of growth may be due to increase in cell turnover and increase in cell migration [24]. Moreover, Epo receptors are present on a variety of non haematopoietic cell types,

including enterocytes, endothelial cells, smooth muscle cells and neurons which are present in the developing bowel [25]. It is possible that Epo acts as a trophic factor on one or more of these cell types during growth and development[26].

Conclusion and Recommendation:

It is concluded that, parenteral administration of human recombinant Epo has haematopoietic and trophic effect on small bowel. Enteral administration of human recombinant Epo is not absorbed in amount sufficient to promote increased erythropoiesis, but associated with trophic effects on the developing small bowel. It is recommended to use enteral human recombinant Epo in treatment of infants suffering defective absorption due to short bowel syndrome .

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دراسة عن التأثير المغذى للإريثروبيوتين البشرى على نمو الأمعاء الدقيقة فى الفئران حديثة الولادة

روميساء على الشربينى و محمود عبد الحميد الغريب
قسم الفسيولوجيا - كلية الطب- جامعة طنطا

بهدف هذا البحث إلى دراسة التأثير المغذى للإريثروبيوتين البشرى على الأمعاء الدقيقة فى الفئران حديثة الولادة.

وقد أجرى هذا البحث على ٢٤ فأرا حديث الولادة- تتراوح أعمارها بين ٤-٥ أيام ووزنها ما بين ٤٠-٦٠ جرام قسمت إلى أربعة مجموعات كل مجموعة مكونة من ستة فئران.

١. المجموعة الأولى: وهى المجموعة الضابطة وقد أعطيت ٢/١ ملم محلول ملح بالفم عن طريق أنبوبة صغيرة.
٢. المجموعة الثانية: وقد أعطيت الإريثروبيوتين البشرى عن طريقة الفم عن طريق أنبوبة صغيرة. بجرعة تساوى ٢٠٠ وحدة/ كجم/ يوم لمدة أسبوع.
٣. المجموعة الثالثة: ولقد أعطيت الإريثروبيوتين البشرى عن طريقة الفم عن طريق أنبوبة صغيرة. بجرعة تساوى ١٠٠٠ وحدة/ كجم/ يوم لمدة أسبوع.
٤. المجموعة الرابعة: وقد أعطيت الإريثروبيوتين البشرى ٢٠٠ وحدة/ يوم لمدة أسبوع بالحقن فى الغشاء البريتونى.

وفى نهاية البحث تم تخدير الفئران ثم وزنت وجمعت عينات الدم من القلب ثم شرحت الفئران واستأصلت الأمعاء الدقيقة وتم قياس طولها ثم حفظت في البرافين للفحص المجهرى.

وقد دلت النتائج على وجود ارتفاع ذو دلالة إحصائية فى وزن الجسم وطول الأمعاء الدقيقة للفئران التي أعطيت الإريثروبيوتين عن طريق الفم وأيضا وجد أن هناك زيادة ذات دلالة إحصائية فى تركيز الإريثروبيوتين فى البلازما ونسبة كرات الدم الحمراء ووزن الجسم فى الفئران المحقونة بالايريثروبيوتين وقد دلت نتائج الفحص المجهرى على زيادة مساحة سطح الغشاء المخاطى المبطن للأمعاء كما زاد طول ونمو الخملات المعوية.

ونستخلص من نتائج هذا البحث أن إعطاء الإريثروبيوتين البشرى عن طريق الحقن يزيد نمو الأمعاء كما يزيد تكوين كرات الدم الحمراء. كما أن إعطاؤه عن طريق الفم فى الفئران حديثة الولادة له تأثير مغذى على الأمعاء الدقيقة ويزيد من نموها ويوصى باستعماله في الأطفال الذين يعانون من نقص فى عملية الامتصاص وذلك بسبب قصر طول الأمعاء الدقيقة.