UNIVERSITI PUTRA MALAYSIA

EFFECTS OF SUBCUTANEOUS AND INTRAVENOUS RECOMBINANT HUMAN ERYTHROPOIETIN TREATMENTS ON BODY IRON IN RATS

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HARETH YAHYA AHMED SHUJAAEDIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, In Fulfilment of the Requirements for the Degree of Master of Science

June 2009

DEDICATION

To my mother, father, my wife, my sons Mohammad and Akrm, my brothers and sisters.

Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman: Professor Rasedee Abdullah, PhD

Faculty: Veterinary Medicine

Recombinant human erythropoietin (rHuEPO) is used widely in clinical practice for correcting anemia related to renal failure, cancer chemotherapy, HIV infection, premature infants, and chronic diseases. Recombinant human erythropoietin is known to affect body iron status that may result in functional iron deficiency (FID). Functional iron deficiency is one of the major causes of insufficient response to rHuEPO. Thus rHuEPO treatment must be accompanied with iron supplementation to avoid iron metabolism disorder and to maintain optimal erythropoiesis. The aim of this study was to compare the effect of subcutaneous (s.c.) with the intravenous (i.v.) rHuEPO administration on the body iron status in rats after short-term and long-term treatments. For the short-term experiment, 20 Sprague-Dawley rats were divided into four groups of 5 rats each; s.c. rHuEPO group (s.c. 150 IU rHuEPO/kg/day), control group for s.c. rHuEPO (s.c. 0.40 - 0.44 mL 0.9% saline solution/rat/day), i.v. rHuEPO group (i.v. 150 IU rHuEPO/kg/day), and

control group for i.v. rHuEPO (i.v. 0.40 - 0.45 mL 0.9% saline solution/rat/day).

The duration of the short-term rHuEPO treatments was 7 days. For the long-term experiment 80 Sprague-Dawley rats were divided into four groups of 20 rats each; s.c. rHuEPO group (s.c. 450 IU rHuEPO/kg/wk), control group for s.c. rHuEPO (s.c. 0.40 - 0.50 mL 0.9% saline solution/rat/wk), i.v. rHuEPO group (i.v. 450 IU rHuEPO/kg/wk), and control group for i.v. rHuEPO (i.v. 0.40 - 0.50 mL 0.9% saline solution/rat/wk). The duration of the long-term rHuEPO treatments was 8 weeks. Blood samples were drawn at the end of the short-term experiment and at wk 2, wk 6 and wk 8 in long-term experiment. Erythrocyte (RBC) counts, haemoglobin (Hb) concentrations, haematocrit (HCT) and blood smears for reticulocytosis were used to detect activation of erythropoiesis caused by rHuEPO treatment. Serum Iron (SI), transferrin saturation (TS), unsaturated iron binding capacity (UIBC), total iron binding capacity (TIBC), serum ferritin (SF), stainable liver iron (LI), and bone marrow iron (BMI) were analysed to determine body iron status (BIST). Erythroc

($p<0.05$) higher in all treatment groups compared to the control groups in both short-term and long-term experiments. The blood smears showed increase in reticulocytes in all treatment groups, while no increase in reticulocytes were observed in the control groups in both short-term and long-term experiments. The SI, TS, and BMI were significantly ($p<0.05$) lower in the treatment groups of short-term and long-term experiments compared to controls. The TIBC was significantly ($p<0.05$) higher in the treatment group of short-term but not in long-term experiment while SF and LI showed no

significant ($p>0.05$) difference between groups except in short-term s.c group. The degree of these changes was greater in the i.v rHuEPO than in the s.c rHuEPO group both for the short-term and long-term experiments. The effect of rHuEPO on iron parameters that suggested increased iron utilization was more apparent in short-term experiment than long-term experiment. In conclusion, this study suggests that among the effects of rHuEPO administration is increasing erythropoiesis through the utilization of serum and storage irons, and that the effect is more pronounced with i.v. than s.c rHuEPO administration particularly in short-term treatment.

Abstrak tesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN PERLAKUAN ERITROPOIETIN MANUSIA REKOMBINAN SUBKUTIS DAN INTRAVENA TERHADAP FERUM BADAN TIKUS

Oleh

HARETH YAHYA AHMED SHUJAAEDIN

June 2009

Pengerusi: Profesor Rasedee Abdullah, PhD

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Erithropoietin rekombinan manusia (rHuEPO) diguna secara luas dalam amalan klinikal untuk rawatan anemia berkaitan kegagalan renal, kemoterapi kanser, jangkitan HIV, anak pramatang, dan penyakit kronik. Eritropoietin rekombinan manusia diketahui dapat memberi kesan terhadap status ferum badan yang mungkin mengakibatkan kekurangan ferum fungsian (FID). Kekurangan ferum fungsian ini merupakan satu daripada penyebab utama kepada kurangnya gerak balas terhadap rHuEPO. Oleh demikian rawatan rHuEPO mesti diiringi dengan penambahan ferum untuk mengelak daripada berlakunya gangguan metabolisme dan untuk menyenggarakan eritropoiesis pada tahap optimum. Tujuan kajian ini ialah untuk membanding kesan pemberian rHuEPO secara subkutis (s.c.) dengan intravena (i.v.) terhadap status ferum badan tikus dalam perlakuan jangka pendek dan jangka panjang. Untuk ujikaji jangka pendek 20 ekor tikus Sprague-Dawley telah dibahagikan kepada empat kumpulan dengan 5 ekor setiap kumpulan: kumpulan rHuEPO s.c (150 IU rHuEPO/kg/hari, s.c.), kumpulan kawalan

untuk rHuEPO s.c. (0.40 – 0.44 mL 0.9% larutan salina/tikus/hari, s.c.), kumpulan rHuEPO i.v. (150 IU rHuEPO/kg/hari, i.v.), dan kumpulan kawalan untuk rHuEPO i.v. (0.40 – 0.44 mL 0.9% larutan salina/tikus/hari, i.v.). Tempoh untuk perlakuan rHuEPO jangka pendek ialah 7 hari. Untuk ujikaji jangka panjang 80 ekor tikus Sprague-Dawley dibahagikan kepada empat kumpulan 20 ekor tikus setiap kumpulan: kumpulan s.c rHuEPO (450 IU rHuEPO/kg/minggu, s.c.), kumpulan kawalan untuk rHuEPO s.c. (0.40 – 0.44 mL 0.9% larutan salina/tikus/wk, s.c.), kumpulan rHuEPO i.v. (450 IU rHuEPO/kg/minggu, i.v.), dan kumpulan kawalan untuk rHuEPO i.v. (0.40 – 0.44 mL 0.9% larutan salina/tikus/minggu, i.v.). Tempoh untuk perlakuan rHuEPO jangka pendek ialah 8 minggu. Darah diperolehi pada penghujung ujikaji jangka pendek dan setiap 2 minggu dalam ujikaji jangka panjang. Kiraan eritrosit (RBC), kepekatan hemoglobin (Hb), hematokrit (HCT) and saput darah untuk retikulosit diguna untuk mengesan pengaktifan eritropoiesis yang disebabkan oleh perlakuan rHuEPO. Ferum serum (SI), ketumpatan transferin (TS), keupayaan pengikatan ferum tak tepu (UIBC), keupayaan pengikatan ferum seluruh (TIBC), feritin serum (SF), ferum hati boleh diwarna (LI), ferum sumsum tulang (BMI) telah dianalisis pada akhir tempoh pengkajian untuk kumpulan tikus perlakuan jangka pendek dan jangka panjang, dengan tujuan menentukan status ferum badan (BIST). Kiraan eritrosit, kepekatan Hb, HCT, dan UIBC, lebih tinggi tererti ($p<0.05$) dalam semua kumpulan perlakuan berbanding kumpulan kawalan untuk kedua-dua ujikaji jangka pendek dan jangka panjang. Saput darah menunjukkan peningkatan retikulosit dalam semua kumpulan perlakuan, sambil tiada retikulosit dilihat dalam kumpulan kawalan bagi kedua-dua ujikaji

jangka pendek dan jangka panjang. Ferum serum, TS, dan BMI lebih rendah tererti ($p<0.05$) dalam kumpulan perlakuan ujikaji jangka pendek dan jangka panjang berbanding kawalan. Keupayaan pengikatan ferum keseluruhan adalah lebih tinggi ($p<0.05$) dalam ujikaji jangka pendek tetapi tidak bagi ujikaji jangka panjang, sambil SF dan LI tidak menunjukkan sebarang perbezaan ($p>0.05$) antara kumpulan kecuali dalam kumpulan s.c. jangka pendek. Tahap perubahan ini adalah lebih tinggi dalam kumpulan rHuEPO i.v. daripada kumpulan rHuEPO s.c. untuk kedua-dua ujikaji jangka pendek dan jangka panjang. Kesan rHuEPO terhadap parameter ferum menyarankan yang peningkatan penggunaan ferum lebih ketara dalam ujikaji jangka pendek daripada jangka panjang. Sebagai kesimpulan, kajian ini menyarankan bahawa antara kesan pemberiaan rHuEPO ialah peningkatan eritropoiesis melalui penggunaan ferum serum dan simpanan, dan kesan ini lebih ketara selepas pemberiaan rHuEPO i.v. daripada s.c., terutama sekali dalam perlakuan jangka pendek

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Lastly, I would like to thank all of those who support me by any kind of help even by a word and they have not been mentioned but surely they are remembered.

I certify that a Thesis Examination Committee has met on 26 June 2009 to conduct the final examination of Hareth Yahya Ahmed Shujaaedin on his thesis entitled "Effects of Subcutaneous and Intravenous Recombinant Human Erythropoietin Treatments on Body Iron in Rats" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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Date: 17 July 2009

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

HARETH YAHYA AHMED SHUJAAEDIN

Date: 1– 7- 2009

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AIDES	acquired immune deficiency syndrome
Bcl-x	B-cell lymphoma-extra large
BIST	Body iron status
BMI	Bone marrow iron
Asn	Asparagines
C	Control
°C	Degrees centigrade
CFU-E	Colony forming unit-erythroid cells
CI_{95}	Confidence interval
CRF	Chronic renal failure
Cys	Cysteine
C-yl	Phospholipase C-yl
dL	Deciliter
DMT-1	Divalent metal transporter 1
DNA	Deoxyribonucleic acid
EPO	Endogenous Erythropoietin
EPOR	Erythropoietin receptor
Fe	Iron
FID	Functional iron deficiency
Fig.	Figure
Hb	Haemoglobin
HCT	Hematocrit
HIF-1	Hypoxia-induced factor-1

hr	Hour
i.m.	Intramuscular
IPRS	Iron regulatory proteins
IU	International unit
i.v .	Intravenous
JAK2	Janus tyrosine kinase 2
kg	Kilogram
L	Litre
LI	Liver iron
MEIA	Microparticle Enzyme Immunoassay Technology
mg	Milligram
µg	Microgram
min	Minutes
ml	Milliliters
µmol	Micromole
N	Nitrogen
n	Number of animals
O	Oxygen
RBC	Total erythrocyte count
rHuEPO	Recombinant human erythropoietin
RNA	Ribonucleic acid
rpm	Revolutions per minute
s.c.	Subcutaneous
Ser	Serine
SF	Serum ferritin

SHC	src homology 2 domain containing
SI	Serum iron
src	Family for oncogenic tyrosine kinase
STAT5	Signal transducer and activator of transcription
sTfR	Soluble transferrin receptors
T	Treatment
TfR	Transferrin receptor
TIBC	Total iron binding capacity
TS	Transferrin saturation
$T_{1/2}$	Half-life
UIBC	Unsaturated iron binding capacity
wk	week