DATE: SEPTEMBER 17, 1984

TO: J. FENNO
N. STEBBING

FROM: D. VAPNEK D.V.

F. Lin Clinical

- -

I HAVE APPENDED THE PHYSICIAN-SPONSORED IND FOR EPO. F. K. LIN AND J. EGRIE HAVE COPIES. A NUMBER OF CLINICIANS HAVE BEEN SUGGESTED AS POSSIBLE CONSULTANTS/CLINICAL PANEL MEMBERS/CLINICAL INVESTIGATORS. THESE INCLUDE DR. ADRIAN KATZ, UNIVERSITY OF CHICAGO; DR. SANFORD KRANTZ, VANDERBILT (VA HOSPITAL); DR. JOHN VAN STONE, DEPT. OF MEDICINE, UNIVERSITY OF MISSOURI; DR. JOHN ADAMSON, HEAD OF HEMATOLOGY, UNIVERSITY OF WASHINGTON, SEATTLE; DR. GARY STRICKER, UNIVERSITY OF WASHINGTON, AND PETER DUKES (PH.D.), UNIVERSITY OF SO. CALIFORNIA.

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THE BEST SOURCE OF FURTHER INFORMATION ABOUT THESE INDIVIDUALS WOULD BE JOAN EGRIE, F. K. LIN AND GENE GOLDWASSER. DR. SANFORD (SANDY) KRANTZ'S NAME COMES UP MOST OFTEN AS A CANDIDATE TO CARRY OUT THE CLINICAL TRIALS. PETER DUKES HAS BEEN WORKING WITH EPO FOR YEARS AND, EVEN THOUGH HE IS NOT AN M.D., COULD PROVIDE VALUABLE INPUT TO THE CLINICAL PANEL. HE HAS BEEN CARRYING OUT THE EPO IN VIVO EXPERIMENTS FOR US.

GENE GOLDWASSER ALSO MENTIONED THAT DIMITROS EMMANOUEL IN ATHENS, GREECE, WOULD LIKE TO BE INVOLVED IN EUROPEAN TRIALS. HE WAS INVOLVED IN THE HUMAN EPO STUDY AT THE UNIVERSITY OF CHICAGO.

DV:PAK

CC: G. B. RATHMANN
F. K. LIN
J. EGRIE

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SUMMARY

Clinical Study of Purified Human Erythropoietin (H-EPO)

Investigator : Joseph Baron, M.D.
Dimitrios Emmanouel, M.D.
Eugene Goldwasser, Ph.D.
Adrian Katz, M.D.

1. Introduction

Recent work in the laboratory of Dr. Goldwasser has resulted in the preparation of highly purified erythropoietin derived from human urine. We wish to study the efficacy of this material as a stimulant of erythropoiesis and its pharmacology in human subjects for the first time.

2. Material

The H-EPO has been diluted in normal human serum albumin, (Albuspan (R), Parke Davis) sterilized by Nuclepore filtration in the University of Chicago Pharmacy Department, and found to be sterile and non-pyrogenic in its final dosage form. The H-EPO is effective in vitro and in vivo (rodents) in stimulating erythropoiesis, and there is no known toxicity in these systems.

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3. Safety

Ward Richter, D.V.M. of the Carlson Animal Research Facility has performed necropsy studies of 8 hamsters (4 injected, 4 controls) given 18x the proposed human daily dose parenterally over a 22 day period. No gross or microscopic change could be attributed to the test material (H-EPO). Hematology samples and blood chemistries drawn from these animals at time of sacrifice were performed at Illinois Institute of Technology Research Institute without significant abnormality detected. The very limited amount of purified H-EPO available precludes toxicity studies in other larger species. There are no human studies previously done with purified H-EPO.

Clinical Protocol (See attached graph)

We plan to do initial studies of H-EPO in 3 or 4 patients with renoprival anemia on chronic hemodialysis. Following a 2-week baseline observation period, we propose to administer 520 units (approx. 6.5µg) intravenously in 1.9ml every 12 hours by slow infusion (over 15-20 minutes) for 7 days. This should achieve an average serum H-EPO level of 50 mu/ml (approximately 3 times baseline levels for these patients). Basic parameters to be followed include only standardized laboratory methods used in daily medical practice (e.g. CBC, reticulocyte count, bone marrow exam, ferrokinetics and red cell mass determinations). No significant risk to the subjects is anticipated. We plan for close observation during the study period.

Efficacy & Significance

Recent studies in dialyzed uremic rodents suggest that H-EPO is likely to be an effective stimulant of erythropoiesis in man. Since the treatment for anemia in renal failure has, in general, been disappointing with other modalities and, because lack of erythropoietin is generally believed to be the single most significant cause of this anemia, it seems especially important to test H-EPO in these patients now that it is available in purified form.

6. Patient Consent

We will use the short form Consent by Subject of Research Project - 1/79 Revision as appended.

7. A protocol previously submitted to the CIC (1973) for study of sheep erythropoietin was approved but not performed pending availability of H-EPO.

8. We plan to submit the accompanying detailed proposal to the Food and Drug Administration as a Physician Sponsored IND pending Clinical Investigation Committee review.

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Physician Sponsored IND

1. Investigator: Joseph M. Baron, M.D.
Associate Professor
Department of Medicine
Section of Hematology
University of Chicago
Chicago, Illinois

2. Name of Drug: Human Erythropoietin (H-EPO)

3. Manufacturing Information:

(c) Human erythropoietin (H-EPO) has been prepared from the urine of patients with aplastic anemia. Details of the source, purification and assay of the material are in the attached reprint of Miyake, et. al. (J. Biol. Chem. 252 (15): 5558-5564, 1977). The final purification and assay of H-EPO have been performed in the laboratory of Dr. Eugene Goldwasser at the University of Chicago, Department of Biochemistry.

The hormone is diluted in Normal Serum Albumin (Human) USP (Albuspan(R), Parke Davis) at a concentration of 276 units/ml. (80,000 units/mg H-EPO protein) to maintain stability and permit appropriate volume for administration. The final dosage form was sterilized in a single batch by Nuclepore filtration in the University of Chicago Pharmacy Department, sealed in vials containing 1.9 ml. of material and frozen (total of 520 units contining approximately 6.5 micrograms of H-EPO protein per vial). The final solution was pyrogen-free and sterile (see attached certificates). The purified H-EPO was negative by Limulus assay prior to dilution in the albumin. The final product was biologically active in vitro and in vivo assays. Long term retention of hormone activity during storage in the frozen state has been demonstrated.

70-80,000 of protein

Human Erythropoietin (H-EPO)
For [illegible] use only
240 units/ml
4[illegible]
Investigator: J. Baron, M.D.
Univ. of Chicago
"Caution: [illegible]-limited by Federal/or United States law to Investigational Use."

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4. Preclinical Information

c. Animal Toxicology

Please see attached report from Ward Richter, D.V.M. of the A.J. Carlson Animal Research Facility at the University of Chicago summarizing a toxicology study in hamsters (22 day course at 18 times the intended human daily per kilogram dose). A very limited supply of the H-EPO precludes a similar study in a second larger species.

e. There are no other animal toxicity data with H-EPO. It is effective in stimulating erythropoiesis in vitro and is not known to be cytotoxic in culture systems. H-EPO stimulates erythropoiesis in rodents in vivo. There are no human toxicology or pharmacology studies with this purified material.

5. Clinical Information

a. Rationale and Objectives

It is proposed to study the pharmacology and efficacy of H-EPO as a stimulant of erythropoiesis in man. Initial trials will be done in a small number of patients with renoprival anemia. Since the lack of erythropoietin is generally believed to be the single most significant cause of this anemic state it seems logical and especially important to test H-EPO in these patients who would appear to have the most to gain from H-EPO. Also, because these patients have lower baseline serum erythropoietin levels than those patients with comparable degrees of anemia due to other causes (e.g. iron deficiency), it is easier to raise the serum H-EPO level with infusion of smaller amounts of the hormone.

Recent rodent data (1,2) and in vitro studies (3) support the likelihood that H-EPO may be effective in stimulating erythropoiesis in human subjects with renoprival anemia.

Additional pharmacologic studies in normal volunteers and patients with anemia of chronic disease would follow if safety and efficacy are demonstrated in the initial trials. Limited amounts of H-EPO currently available preclude large scale studies.

5. b. References

1. Anagnostou, A., Barone, J., Kedo, A., and Fried, W.: Effect of Erythropoietin Therapy on the Red Cell Volume of Uremic and Non-Uremic Rats.

Brit. J. Haemat. 37: 85, 1977.

5. b. References (Cont'd)

2. Van Stone, J.C. and Max, P.: The Effect of Erythropoietin on the Anemia of Uremia.

American Society of Nephrology (abstract - 11th Annual Meeting; p. 100A) (See attached copy).

3. Urabe, A., Chiba, S., Kosaka, K., Takaku, F.: Response of Uremic Bone marrow Cells to Erythropoietin In Vitro.

Scand. J. Haematol. 17:335-340, 1976.

4. Fisher, J.W.: Erythropoietin: Pharmacology, Biogenesis and Control of Production.

Pharmacological Reviews, 24(3): 459-508, 1972.

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5c. Clinical Protocol

(See attached graph).

a. Patient population

1. Initial studies of H-EPO will be performed in 3 or 4 adult individuals with anemia of chronic renal failure who are hemodialyzed three times weekly and are hematologically stable. Patients of either sex with or without kidneys will be eligible. Ideal candidates will not have a red cell transfusion requirement or be taking androgens to stimulate erythropoiesis.

Patients will be evaluated prior to H-EPO administration to exclude other contributing causes of anemia, to permit correction of conditions which might preclude response to H-EPO (e.g. limiting nutrient(s)), and to carefully delineate the pre-treatment state of erythropoiesis.

b. Protocol

Baseline Studies will include:

1. Serial CBC, platelets, reticulocyte count and differential.
2. Stools for blood.
3. Bone marrow aspiration and biopsy to determine cellularity, status of iron stores, number of normoblasts/1000 nucleated marrow cells.
4. Serum folic acid, B_{12}, serum iron, TIBC, serum ferritin, Coombs' & T4/FTI.
5. Chromium^{51} red cell mass and blood volume.
6. Ferrokinetics - to include plasma iron turnover (PIT) and incorporation of Fe^{59} into red cells (% iron utilization).
7. EKG, Chest X-ray, SMA-17, prothrombin time and partial thromboplastin time.

8. Serum H-EPO level - by radioimmunoassay (technique available in Dr. Goldwasser's laboratory).

9. Urinary H-EPO determinations (in patients with urine output).

Patients on study will be hospitalized for intravenous infusions of H-EPO as described below. They will be housed in the Clinical Research Center at the University of Chicago Hospitals. All acute hospital facilities and personnel are available to deal with any untoward reaction.

H-EPO will be given by intermittent slow intravenous infusion of 520 units (in 1.9ml volume over 15-20 minutes) every 12 hours. (See attached sheet on pharmacokinetic considerations). This dose aims to raise the average serum H-EPO concentration to 50mu/ml (approximately 3x the average normal level of 16.5mu/ml). Patients with the anemia of chronic renal failure have serum H-EPO levels at (by bioassay) or slightly above (by radioimmunoassay) the normal in baseline conditions. Calculations of the pharmacokinetics are based upon the assumption of a plasma T1/2 of approximately 12 hours (ref. 4) lack of significant urinary loss in these patients and no loss by dialysis (molecular weight is too high). It is proposed to treat for 7 days (14 doses) with a subsequent observation period of 2 weeks.

Parameters to be monitored during and after the treatment period include:

1. CBC, platelet, reticulocytes every other day using smallest possible amounts of blood so as not to obscure any significant red cell mass increase.

2. Serum H-EPO levels daily with additional determinations after the first and last injections to evaluate disappearance time from plasma (radioimmunoassay is performed on samples of < 1ml serum).

3. Repeat SMA-17 on days 3, 7, and 21.

4. Repeat ferrokinetics and bone marrow studies - day 10.

5. Repeat Cr^{51} red cell mass and blood volume - day 21.

6. Daily 24 hr. urine collection for H-EPO assay (as available).

Pharmacokinetics (prepared by Dr. David Kornhauser - Department of Medicine, Section of Clinical Pharmacology; University of Chicago).

Assumed

1. First order kinetics - $C = C_o e^{-Kt}$

   At steady state - peak C^p and through C^t concentrations are identical following every dose.

2. Time course: $C^t = C^p e^{-kT}$ where T=dosing interval

3. Average concentration (c) over dosing interval T = $\dfrac{\int_o^T C^p e^{-Kt}}{T}$

   Average concentration (c) = $\dfrac{C^p e^{-Kt}}{KT}\Big|_o^T$

   $= \dfrac{C^p}{KT}\left(-e^{-Kt}\Big|_o^T\right)$

   $= \dfrac{C^p}{KT}\left(1-e^{-KT}\right)$

   For T = 12 hr. = 1 T1/2
   Dose = 520 units q 12 Hrs.

   Average concentration (c) = $\dfrac{C^p}{KT1/2}\left(1-e^{-KT1/2}\right)$

   $= \dfrac{C^p}{.693}\left(1-e^{-\frac{1}{693}}\right) = \dfrac{C^p}{.695}\left(1-1/2\right)$

$$\text{Avg. } C = \frac{C^p \cdot 1/2}{.693} =$$

$$\text{Avg. } C = .727C^p$$

4. For Avg. C of 50 mu/ml

$C^p = 69.3$ mu/ml

$C^t = \frac{C^p}{2} = 34.65$ mu/ml

5. Expect 96.875% steady state level at 60 hrs.

6. Distribution Space Estimate for Dose Calculations

a. For uremic patient: 70% Body Wt. = Water
ECF = 1/3 of total body water
70Kg subject
Space = (0.7)(.33)(70) $\cong$ 15 Liters

b. Clearance = K Vol.

K x t 1/2 = 0.693; t 1/2 = 12 hrs. (ref. 4)
K x 1/2 day = .693
K = 1.386/day

Clearance = 1.386 x 15,000 $\cong$ 20,800 ml/day

c. Clearance x Steady State Concentration = Infusion rate.

d. For steady State Concentration of 50 mu/ml
need 20,800 ml/day x 50 mu/ml =

1040 Units/day
to be infused

THE EFFECT OF ERYTHROPOIETIN ON THE ANEMIA OF UREMIA. John C. Van Stone and Paul Max*. Dept. of Medicine, Truman VAH and University of Missouri, Columbia, Missouri.

Although erythropoietin (EPO) is a potential therapeutic agent in anemia of uremia, previous studies suggest that there is resistance to its action in uremia which may limit its usefulness.

Four groups of 5 rats each, were peritoneally dialyzed for 12 days: (1) surgically anephric rats given 1 ml saline daily (STX), (2) Anephric rats receiving 2 units of EPO/day (STX+EPO), (3) Control sham operated saline injected rats (C), (4) Control rats given 2 units EPO (C+EPO). Plasma iron turnover (PIT) and red cell mass were determined after 8 days and RBC Fe^{59} uptake, marrow nucleated RBC, HCT and BUN determined at 12 days.

	STX	STX+EPO	C	C+EPO
BUN (mg/dl)	107*	106*	25	18
PIT (mg/Kg/day)	1.01*	1.24*	1.56	1.92
RBC Fe^{59} UPTAKE (%)	48*	71*	74	70
NUCLEATED RBC/ FEMUR ($x10^7$)	0.64*	2.12*	2.22	2.65
HCT (%)	19*	26**	42	44
RBC MASS (ml/Kg)	14.6*	25.6**	24.0	31.4

*p < 0.05 compared to C

Our data indicated that [illegible] the marked bone marrow depression and significantly improves the anemia present in the uremic rat and suggests EPO will be a useful [illegible] for the treatment of anemia of chronic renal failure.

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Period (2 week baseline)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Red cell mass (Cr^{51})	x																						x
Ferrokinetics	———																						
Bone marrow										x													
EVA-17	x	x				x		x															x
Serum H-EPO	x	x	x			x		x		x		x		x		x		x		x		x	x
CSC, retic	xxxxx					x		x		x		x		x		x		x		x		x	x
H-EPO Therapy	———																						

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CLINICAL PROTOCOL H-EPO

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