Original Article

The Metabolism of Erythropoietin in the Normal and Uraemic Rabbit

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Abstract. The metabolism of recombinant human erythropoietin (rHuEpo) labelled with 125I has been investigated in five normal and nine 5/6-nephrectomised rabbits. The plasma erythropoietin half-life was significantly prolonged at 5.1 ± 1.2 h (mean ± SD) in the 5/6-nephrectomised rabbits, compared to 3.0 ± 0.4 h in sham-operated controls (P<0.001). The disappearance of 125I-labelled rHuEpo is biphasic. Examination of serum by fast protein liquid chromatography (FPLC) following administration of 125I-labelled rHuEpo by FPLC showed a single peak of radioactivity in all rabbits except two of the nephrectomised group. In serum from both of these animals a second labelled peak was found, corresponding to material of MW 200 000–250 000 D. We conclude that the 5/6-nephrectomised rabbit provides a stable model for the study of hormonal metabolism in chronic renal failure.

Key words: Chronic renal failure; Erythropoietin; Pharmacokinetics

Introduction

Erythropoietin is the principal regulatory hormone of the circulating red cell mass. It is a glycoprotein of molecular weight 30 400 D [1] which is synthesised in the kidney and is normally present in serum at picomolar concentration. Erythropoietin is metabolised in the liver, kidney and bone marrow [2–4] but little is known about the effect of uraemia on its metabolism.

In previous investigations of erythropoietin metabolism an estimation of plasma half-life (t1/2) was made following administration of impure erythropoietin preparations and determination of serum values by bioassays which were subject to error [5–10]. These and other studies, employing a variety of animal models, suggested that the plasma clearance of erythropoietin is biphasic [4–15].

Neither total nephrectomy nor ligation of renal pedicles results in an ideal model for chronic renal failure, a condition in which some renal function remains. A useful long-term model of chronic uraemia is provided by the 5/6-nephrectomised rabbit and we have used this model to investigate the metabolism of radiolabelled recombinant human erythropoietin (rHuEpo) in chronic uraemia.

Materials and Methods

Rabbit Model of Chronic Renal Failure

Mixed breed rabbits, both male and female, mean weight 3.95 (±0.92) kg, were 5/6 nephrectomised in two stages [16]. All procedures were carried out within the regulations and guidelines of the Animals (Scientific Procedures) Act 1986. General anaesthesia was achieved using 0.3 ml/kg body weight ‘Hypnorm’ (fentanyl citrate, 0.3 mg/ml and fluanisone, 10 mg/ml; Janssen Pharmaceuticals, Oxford) and 0.5 mg diazepam intramuscularly. The upper and lower poles of the left kidney were removed through a flank incision and electrocautery was used to obtain haemostasis. Subcutaneous buprenorphine was used for postoperative analgesia. Two weeks later, the entire right kidney was removed under similar conditions. Control animals were subjected to a two-stage sham
concentrations. There were no significant changes in their mean body weight had decreased from a preoperat-
ive level of 3.5 ± 0.8 kg to 3.0 ± 0.6 kg (P < 0.02, Student's t test), serum creatinine had increased from 83 ± 19 umol/1 (4.86 ± 2.73 mmol) to 430 ± 241 umol/1 (4.86 ± 2.73 mmol) and haemoglobin had decreased (P < 0.01) during the next 24 h were collected for safe disposal.

Determination of the Half-life of 

A 23-gauge Butterfly (Abbot, Ireland) cannula was inserted into an ear vein of the subject rabbit and 1.5 ml of 125I-labelled erythropoietin containing 2–3 μCi (74–111 K Bq) in isotonic saline injected slowly over 15 s, followed by 2 ml of 0.9% sodium chloride to flush the cannula. Serial 2-ml blood samples were drawn from a vein on the opposite ear at 5, 20, 45, 90, 180, 270 and 360 min. Samples were allowed to clot at room temperature for 1 h, centrifuged at 3000 r.p.m. for 5 min, and a 1-ml aliquot of serum removed. Total radioactivity in 1 ml of serum from the serial blood samples was measured in an NE 1600 gamma counter (Nuclear Enterprises, Edinburgh, Scotland). All urine and faeces eliminated during the next 24 h were collected for safe disposal.

The plasma t1/2 of circulating 125I-erythropoietin was calculated using the method of Emmanouel et al [4], from regression lines.

Results

All nine of the 5/6-nephrectomised rabbits appeared healthy and had normal eating habits at the time of study. Their mean body weight had decreased from a preoperative level of 3.5 ± 0.8 kg to 3.0 ± 0.6 kg (P < 0.02, Student's t test), serum creatinine had increased from 83 ± 19 μmol/l (0.94 ± 0.21 mg/100 ml) to 430 ± 241 μmol/l (4.86 ± 2.73 mg/100 ml) (P < 0.01) and haemoglobin had decreased from 11.9 ± 0.6 g/dl to 8.9 ± 0.8 g/dl (P < 0.01). The 5/6-nephrectomised rabbits survived for a mean of 5.4 ± 2.2 months with stable serum creatinine and haemoglobin concentrations. There were no significant changes in weight, serum creatinine, or haemoglobin in the five sham-operated animals.

Plasma t1/2 of Erythropoietin in Normal and 5/6-Nephrectomised animals

Fifteen pharmacokinetic profiles were obtained from nine uraemic rabbits, one of which was studied twice, and five control sham-operated rabbits. The metabolism of 125I-erythropoietin is biphasic. The initial α phase represents the distribution of the labelled hormone into the tissue space and the slower β phase the clearance of 125I-erythropoietin from the circulation (see Fig. 1). Emmanouel et al [4] have shown that the plasma t1/2 for the α phase for a single bolus dose is similar to the t1/2 determined in the steady-state conditions following intravenous infusion of labelled erythropoietin for 2 h. We therefore calculated plasma t1/2 values from the β phase regression equation. The plasma t1/2 in uraemic rabbits was significantly prolonged to 5.1 ± 1.2 h compared with 3.0 ± 0.4 h in normal rabbits (P < 0.001).

Gel Filtration Chromatography of Serum Samples Following Administration of 125I-Erythropoietin

The 125I-labelled erythropoietin administered to the experimental animals was free from aggregates of the hormone, as determined by reverse-phase liquid chromatography. Serum samples obtained from the animals following administration of the labelled hormone were subjected to gel filtration chromatography using Superose 12 on a Pharmacia fast protein liquid chromatography (FPLC) system [17]. In this system proteins are eluted in descending order of their molecular weight, and under the conditions used 125I erythropoietin is eluted after 145 minutes. Serum from all five normal and seven of the nine uraemic animals contained 125I-labelled erythropoietin which eluted at the expected time, see Fig. 2a. However in a further two uraemic animals labelled material was present as two peaks, see Figs 2b and 2c. The extra peak eluted at 120 minutes, which corresponds to a molecular weight of 200 000–250 000 D. The pharmacokinetic curves for these two animals did not differ from the others in the nephrectomised group. In all the animals the chromatographic elution profile was independent of the time of sampling.

The high molecular weight peak found in two of the nephrectomised rabbits suggests that some of the 125I-labelled erythropoietin was bound to a serum protein in these animals. This may cause an alteration in the calculated mean t1/2. However when data from these two animals were omitted the calculated mean t1/2 was 5.0 ± 1.2 h, similar to the value for the group of nine nephrectomised animals.
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Fig. 1. Decay curve for disappearance of $^{125}$I erythropoietin in a normal rabbit.

Incubation of $^{125}$I-Erythropoietin with Rabbit Serum In Vitro

Rabbit sera incubated with $^{125}$I-erythropoietin for 2 hours at room temperature were analysed using gel filtration chromatography. Two peaks were present in the serum of both animals which had previously been shown to form these products in vivo. None of the sera obtained from the other rabbits produced a second peak, once again in agreement with the results in vivo.

Discussion

The rate of removal of circulating erythropoietin in experimental animals has been measured using both endogenously produced erythropoietin following hypoxic stress [7,9-11] and exogenously administered material [4-14,18]. The former approach suffers from the disadvantage that increased production of erythropoietin continues after removal of the hypoxic stimulus, thereby leading to over-estimates of plasma erythropoietin $t_{1/2}$ values. Following administration of exogenous erythropoietin there is an initial rapid distribution of hormone (a phase) in a volume which is approximately 1.5-2 times the plasma volume [7,8,12,21]. Calculation of the plasma erythropoietin $t_{1/2}$ is based on data from the second or $\beta$ phase, and failure to exclude data from the first phase leads to underestimates of the plasma clearance rates.

Data obtained following equilibration within the distribution space in normal animals indicate plasma erythropoietin $t_{1/2}$ values of 1.5–3.5 h in the rat [4,5,7,9,11,12,14,15], 9–10.5 h in the dog [6,13] and 9 h in the sheep [18]. Roh et al [8] reported $t_{1/2}$ values of 8 and 10.25 h in normal rabbits, considerably longer than the $t_{1/2}$ of 3.0 ± 0.4 h found in the present study. This earlier work was carried out before highly purified labelled erythropoietin was available. Most previous reports have been based on assays of bioactivity which cannot differentiate between endogenous and exogenously administered erythropoietin or have used impure preparations of radio-labelled
erythropoietin. Emmanouel et al [4] using pure $^{125}$I-labelled human urinary erythropoietin, found a plasma erythropoietin $t_\frac{1}{2}$ value of $3.5 \pm 0.2$ h in normal rats, in good agreement with earlier estimates, and a prolonged value of $4.4 \pm 0.3$ h in rats in which the renal pedicles had been ligated. The rate of clearance of $^{125}$I-labelled human erythropoietin was compared following a single bolus injection and after discontinuation of a constant infusion to equilibrium. Equivalent values for $t_\frac{1}{2}$ were obtained using the two methods when data points for the initial phase were excluded. Making similar assumptions and using $^{125}$I-labelled rHuEpo, we have found a $t_\frac{1}{2}$ of $3.0 \pm 0.4$ h in the normal rabbit and a considerably extended value of $5.1 \pm 1.2$ h in the 5/6-nephrectomised rabbit. Comparable increases in $t_\frac{1}{2}$ in uraemia have been found in the rat [4,9,11] and the dog [13].

Comparative studies have not been performed in humans with chronic renal failure and normal healthy volunteers. The pharmacokinetics of rHuEpo in patients with chronic renal failure have been studied and a similar multieponential decay pattern exists [19-22]. The plasma $t_\frac{1}{2}$ in haemodialysis patients ranged from 8 h to 12 h [19-21] and from 6 to 10 hours in CAPD patients [22]. Urabe et al [23] studied the single-dose pharmacokinetics in eight healthy volunteers, but this study has methodological deficiencies and does not include the corresponding studies in patients with chronic renal failure. They reported a $t_\frac{1}{2}$ of 12 h in normal volunteers. The loss of urinary excretion is insufficient to explain the total increase in plasma erythropoietin $t_\frac{1}{2}$. Normally 4%-7% of the circulating erythropoietin is excreted by the kidney in dogs [6] and approximately 10% is eliminated by this route in humans [24]. Therefore the prolongation of the half-life by approximately 70% in uraemic rabbits is much greater than would be expected. The kidney metabolises many proteins and polypeptides [25] so it may catabolise as well as excrete erythropoietin. In the 5/6-nephrectomised animal any such catabolic activity would be severely curtailed. Impaired hepatic function due to uraemia may also have contributed to the prolongation of erythropoietin plasma $t_\frac{1}{2}$ in the nephrectomised animals. It is of interest that Mladenovic et al [18] using an 11/12-nephrectomised sheep model found no detectable effect on plasma $t_\frac{1}{2}$, suggesting that the main sites of erythropoietin metabolism are extrarenal. Dialysis of the sheep may have improved liver function and led to increased catabolism of erythropoietin.

It has been shown that the carbohydrate component of the glycoprotein protects erythropoietin from catabolism as asialoerythropoietin is rapidly removed from the circulation [14,15] mainly by hepatic degradation [15]. It is possible that the longer plasma $t_\frac{1}{2}$ of rHuEpo in uraemic rabbits is in part due to a delay in the cleavage of the carbohydrate moiety from the hormone in uraemic serum. However this is contradictory to the view expressed by Shannon et al [26] who demonstrated the presence of elevated concentrations of glycosidases in the serum of patients with chronic renal failure.

In the present investigation there was no evidence for fragmentation of the administered $^{125}$I-erythropoietin in either normal or uraemic animals. It is interesting that serum from two of the uraemic animals contained high-molecular-weight $^{125}$I-labelled material. It is not clear whether this represents an aggregated form of erythropoietin or erythropoietin bound to another plasma protein. The estimated molecular weight of this material is 200 000-250 000 D and the absence of multiple labelled peaks suggests that it is not one of a polymeric series of $^{125}$I-erythropoietin, but rather that it is $^{125}$I-erythropoietin bound to a specific plasma protein. Clearly individual variation exists in the handling of administered erythropoietin in uraemic animals. It would be of interest to establish whether such variation exists in man.

In conclusion, the 5/6-nephrectomised rabbit provides a useful model for chronic renal failure. The animals tolerate the uraemia well, show relatively stable blood chemistry and survive for several months, albeit with some weight loss. This study has confirmed that uraemia causes an increase in plasma erythropoietin $t_\frac{1}{2}$ and that the plasma clearance is biphasic. The impairment of hepatic function may be a major factor in the reduced catabolism of erythropoietin. The prolonged plasma erythropoietin $t_\frac{1}{2}$ found in the uraemic animals may have a direct implication for patients with chronic renal failure in whom impaired catabolism of administered rHuEpo would be beneficial.

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References

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