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Intraperitoneal Administration of Recombinant Human Erythropoietin in Uremic Animals

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Previous protocols of administration of recombinant human erythropoietin to patients with end-stage renal disease have been by the i.v. route. Because this method would be impractical for the continuous ambulatory peritoneal dialysis patient, we designed an i.p. dosing protocol in uremic rabbits to examine whether significant amounts of this hormone could be absorbed from the peritoneal cavity. Our results demonstrate that almost all of the erythropoietin is absorbed (or adsorbed) during a prolonged dwell when administered undiluted by dialysate.

body weight of unlabelled hormone (recombinant human erythropoietin 2000 U /mL in sterile buffered solution, 2.5 mg/mL human albumin USP; Ortho Pharmaceuticals Corp., Raritan, NJ).

Four protocols of i.p. administration of erythropoietin were used:

Group 1 (n = 6): Empty Peritoneal Cavity Model. In this group, radiolabelled and unlabelled hormone were dissolved in 10 mL of saline vehicle and instilled in the peritoneal cavity via a Veress needle, where it was left for 24 h. Blood was sampled for radioactive counts at 1,2,3, 6, and 24 h. At the end of the study, the animals were sacrificed and the radioactive erythropoietin remaining in the peritoneal cavity recovered.

This procedure was designed to simulate the clinical setting where rHuEpO could be instilled at the end of a session of intermittent peritoneal dialysis (IPD) and left for 24 h or more. It also partially reflects the setting where continuous ambulatory peritoneal dialysis (CAPD) patients could alter their dialysis regimen and instill rHuEpO in the empty (dialysate-free) peritoneal cavity before going to sleep for the night. Absorption from the peritoneal cavity could then proceed overnight, for as long as 12 h.

Group 2 (n = 4): CAPD Dwell Model. The same doses of radiolabelled and nonlabelled hormone were dissolved in 35 mL/kg body weight of 1.5% Dianeal (Baxter), infused into the peritoneal cavity via a Veress needle and left for a 6-h dwell. Blood was sampled for radioactivity at 1, 2, 3, and 6 hours. At the end of 6 h, the animals were sacrificed and the volume of dialysate remaining in the peritoneal cavity was determined (see below). In addition, the radioactivity in this residual volume was also measured. This protocol was designed to simulate the typical 6-h dwell time of peritoneal dialysate during CAPD.

Group 3 (n = 5): In this group, hormone was infused into the empty peritoneal cavity as in Group 1 but left for only 1 h. At the end of the hour, 35 mL/kg body weight of dialysate (1.5% Dianeal) was infused and left for an additional 6 h. Blood was sampled at 1, 2, 3, 4, and 6 hours after dialysate infusion. At the end of the 6h dialysate dwell, the animals were sacrificed and the volume of fluid remaining and the radioactivity were measured as in Group 2.

The protocol was designed with the expectation

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The anemia of chronic renal failure is usually hypoproliferative, normochromic, and normocytic.

The primary cause of anemia in this setting is the relatively insufficient secretion of the hematopoietic hormone erythropoietin by the failing kidneys (1-5). The recent availability of human erythropoietin produced by recombinant DNA technology has allowed its use in several human trials (6-9). In these studies, recombinant human erythropoietin (rHuEpO) was administered intravenously to hemodialysis patients. The purpose of the present study was to determine whether erythropoietin could be absorbed from the peritoneal cavity in uremic animals.

METHODS

Uremia was induced in female New Zealand white rabbits by a two-phase operative procedure as previously described (10). In all studies, 1 μCi/kg body weight of radiolabelled rHuEpO, that is, ¹²⁵I-rHuEpO

{ (3-[¹²⁵I]iodotyrosyl) erythropoietin, human; Amersham, UK } was used in combination with 400 U /kg

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that absorption of erythropoietin in the CAPD group is reduced as a result of dilution of hormone by dialysate and the consequent reduction in the concentration gradient between peritoneal cavity and plasma. By allowing the hormone to dwell in the peritoneal cavity undiluted for 1 h, it was anticipated that overall absorption could be optimized without altering the CAPD regimen to a great extent.

Group 4 (n = 5): This protocol was identical to that in Group 3, except that the hormone was allowed to dwell in the peritoneal cavity undiluted for 2 h instead of 1. The dwell was followed by infusion of dialysate for 6 h, so that the total duration of the study was 8 h. Blood was drawn for radioactive counts at each hour. At the end of the experiment, the animals were sacrificed and the volume of fluid left in the peritoneal cavity was measured and sampled for radioactivity.

This protocol was designed with the same rationale as that for Group 3. In this case, we wanted to determine whether a 2-h dwell of undiluted hormone would achieve better absorption than a 1-h dwell of undiluted hormone.

Measuring residual volume in the peritoneal cavity directly at the time of sacrifice may underestimate the true residual volume because of loculation of dialysate. Therefore, the volume of fluid that was left in the peritoneal cavity at the end of each experiment was measured as follows: After sacrificing the animals, the abdomen was opened and a sample of fluid withdrawn for measurement of radioactive erythropoietin. Following that, 100 mL of saline was infused to dilute the residual volume of dialysate. After ensuring good admixture of these two volumes, another sample was withdrawn for radioactive counts. The residual volume at the end of the study could then be calculated as follows:

$$V_{res} \text{ (mL)} = \frac{EpO_{dil}}{EpO_{res} - EpO_{dil}} \times 100$$

where V_{res} equals residual volume at the end of dialysis (mL); EpO_{res} equals concentration of EpO in residual volume (cpm/mL); EpO_{dil} equals concentration of EpO in residual volume after dilution with 100 mL saline (cpm/mL).

The amount of erythropoietin absorbed was calculated by measuring the recovery of radioactivity in the peritoneal cavity. A known amount of radioactive

counts was instilled in the peritoneal cavity. At the time of sacrifice, the residual volume and counts per volume were measured (see above). This radioactivity was subtracted from the total initially infused, and the difference expressed as a percentage of the total amount administered.

It is recognized that the amount of erythropoietin leaving the peritoneal cavity may not necessarily equal the amount appearing in plasma. Some of the hormone may distribute across the mesothelium, interstitium, and lymphatics, or deposit in nonvascular spaces such as bone marrow.

All blood and peritoneal dialysate radioactive counts were performed on acid-precipitated protein fractions. Linear correlation analysis was performed on an Epistat program. Between-groups statistical testing was not performed because of the high probability of Type II error.

RESULTS

The characteristics of the 20 animals submitted for study are shown in Table 1. That the rabbits were sufficiently uremic can be seen by the increased urea and creatinine concentrations postoperatively.

GROUP

1

The mean (±SD) erythropoietin absorption from the empty peritoneal cavity at the end of 24 h was 97.5 ±0.4% of the administered dose. This finding indicates that almost all of the instilled erythropoietin was absorbed during that time.

GROUP

2

The average amount of radiolabelled rHuEpO absorbed over the 6-h dwell was 60.0 ±7.4%. The average amount of dialysate absorbed over the same period was 46.1 ±9.2%.

GROUP

3

The mean erythropoietin absorption at the end of 7 h was 59.0 ±3% of the instilled amount, suggesting that the 1-h undiluted dwell time for the hormone did not measurably change its absorption.

TABLE 1
Characteristics of Rabbits Receiving Intraperitoneal Erythropoietin

	Rabbits (n = 20)					
	Weight (kg)	HCT (%)	Total protein (g/L)	Blood glucose (mmol/L)	Blood urea (mmol/L)	Creatinine (μmol/L)
Presurgery	3.5 ± 0.4	42 ± 2	6.1 ± 0.2	7.7 ± 1.5	8.1 ± 1.1	120 ± 15
Postsurgery	3.3 ± 0.4	37 ± 3	6.6 ± 0.3	7.1 ± 1.4	22.5 ± 8.5	418 ± 126

TABLE 2

Percentage Absorption of Intraperitoneal Erythropoietin

Group 1	(rHuEpO in dry peritoneal cavity) mean (\pm SEM) absorption (24 h) = $97.5 \pm 0.4\%$
Group 2	(rHuEpO plus dialysate for 6-h dwell) mean absorption (6 h) = $60.0 \pm 7.4\%$
Group 3	(rHuEpO alone for 1 h; add dialysate for 6-h dwell) mean absorption (7 h) = $59.0 \pm 3\%$
Group 4	(rHuEpO alone for 2 h; add dialysate for 6-h dwell) mean absorption (8 h) = $76.0 \pm 5.0\%$

GROUP 4

The average absorption of erythropoietin after a 2-h dry period and 6-h dialysate dwell was $76.0 \pm 5.0\%$. During this period, the average absorption of dialysate was $62 \pm 9.4\%$. These results are summarized in Table 2.

The relationship between absorption of $^{125}\text{IrHuEpO}$

and absorption of dialysate in the 14 rabbits comprising Groups 2, 3, and 4 is shown in Figure 1. There was a strong positive correlation with $r = 0.89$ and $p < 0.001$, suggesting that the absorption of rHuEpO was linked to absorption of dialysate.

DISCUSSION

Previous studies in hemodialysis patients have demonstrated a dramatic correction of anemia when rHuEpO is administered intravenously three times weekly at the end of dialysis (6-8). This regimen would prove inconvenient for those patients on CAPD. The dosing protocols as currently practiced would require these patients to come to hospital three times a week and receive an intravenous infusion. Therefore, we wondered whether rHuEpO could be administered along with the peritoneal dialysate in a manner similar to the i.p. administration of insulin (11-12). For this method to be effective, however, the peritoneal cavity would have to be capable of transporting this large molecular weight protein.

This study demonstrates that erythropoietin is capable of being absorbed from the peritoneal cavity in significant amounts. From the protocols used, it appears that maximal absorption of the hormone occurs when it is instilled in the peritoneal cavity undiluted by dialysate and allowed to dwell for 24 h.

The mechanism by which rHuEpO is absorbed is not clear. There was a strong correlation between absorption of hormone and absorption of dialysate (Figure 1). This relationship could be best explained by postulating that transport of this large molecular weight protein (30400 daltons) is significantly influenced by convective flux. Figure 1 also demonstrates that when no dialysate absorption occurs (that is, $x = 0$), the percentage absorption of erythropoietin

would be expected to be only 35% of the given dose. In addition, if net ultrafiltration were to occur, absorption of this hormone would be compromised to a greater extent. The peritoneal lymphatics may be important in absorption of this large molecular weight protein (13), but their role in this regard needs to be further defined.

To ensure optimal absorption, the hormone should be instilled in the peritoneal cavity without dialysate and left for a prolonged dwell. For patients on intermittent peritoneal dialysis, rHuEpO could be infused at the end of a dialysis session and simply left in the empty peritoneal cavity until the next visit. However, it remains to be seen whether twice weekly administration of erythropoietin with intermittent peritoneal dialysis will as effectively correct anemia as does thrice weekly infusions with hemodialysis. To best reconcile the kinetics of i.p. transport of rHuEpO as demonstrated in this study with the CAPD regimen, the hormone should be administered undiluted by dialysate. Even allowing one or two hours for absorption of undiluted hormone (Group 3 and 4) before adding dialysate led to significant wastage of this expensive product of recombinant technology. A modified CAPD regimen whereby all the exchanges are performed during waking hours and the peritoneal cavity is left empty overnight may be useful in this regard. Patients on CAPD could be changed to night dry to allow instillation of hormone undiluted by dialysate. It could then be absorbed overnight. Furthermore, when dialysate is infused in the morning, there will be further opportunity for absorption of hormone during the first dwell, although it is anticipated that lesser amounts will be absorbed. The frequency of hormone administration to maintain adequate hematocrits in this population needs to be determined.

In summary, we have demonstrated that rHuEpO is

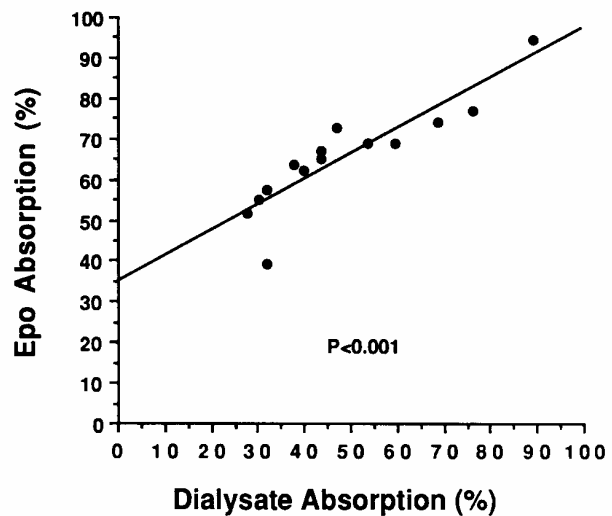


Figure 1—Absorption of erythropoietin from the peritoneal cavity as a function of absorption of dialysate. There is a strongly significant correlation between the two ($r = 0.89$).

capable of being absorbed from the peritoneal cavity of uremic animals in significant amounts. Studies are in progress to determine whether absorption in uremic humans occurs to a similar extent.

REFERENCES

1. Jacobson LO, Goldwasser E, Fried W, *et al.* Role of the kidney in erythropoiesis. *Nature*. 1957; 179:633-634.
2. Loge JP, Lange RD, Moore CV. Characterization of the anemia associated with chronic renal insufficiency. *Am J Med*. 1958; 24:4-18.
3. Naets JP. Role of the kidneys in erythropoiesis. *J Clin Invest*. 1960; 39:102-110.
4. Adamson JW, Eschbach JW, Finch CA. The kidney and erythropoiesis. *Am J Med*. 1968; 44:725-733.
5. Eschbach JW, Adamson JW. Anemia of end-stage renal disease (ESRD). *Kidney Int*. 1985; 28:1-5.
6. Winearls CG, Oliver DO, Pippard MJ, *et al.* Effect of human erythropoietin derived from recombinant DNA on the anemia of patients maintained by chronic haemodialysis. *Lancet*. 1986; 2:1175-1178.
7. Zins B, Drueke T, Zingraff J, *et al.* Erythropoietin treatment in anaemic patients on hemodialysis. (Letter) *Lancet*. 1986; 2:1329.
8. Eschbach JW, Egrie JC, Downing MR, *et al.* Correction of the anemia of end-stage renal disease with recombinant human erythropoietin. *N Engl J Med*. 1987; 316:73-78.
9. Moia M, Mannucci PM, Vizzotto L, *et al.* Improvement in the haemostatic defect of uraemia after treatment with recombinant human erythropoietin. *Lancet*. 1987; 2:1227-1229.
10. Gotloib L, Crassweller P, Rodela H, *et al.* Experimental model for studies of continuous peritoneal dialysis in uremic rabbits. *Nephron*. 1982; 31:254-259.
11. Wideroe T, Smeby LC, Berg KJ, *et al.* Intraperitoneal 125I insulin absorption during intermittent and continuous peritoneal dialysis. *Kidney Int*. 1983; 23:22-28.
12. Mactier RA, Khanna R. Intraperitoneal insulin in diabetic CAPD patients. (Editorial) *Int J Artif Organs*. 1988; 11:9-12.
13. Mactier RA, Khanna R, Twardowski Z, *et al.* Contribution of lymphatic absorption to loss of ultrafiltration and solute clearances in continuous ambulatory peritoneal dialysis. *J Clin Invest*. 1987; 80:1311-1316.