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Continuous versus pulsatile administration of erythropoietin (EPO) via the uterus in anemic rats

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Abstract

We have recently discovered that peptides are absorbed biologically intact from the rat uterus. The purpose of this investigation was to assess whether EPO, a large polypeptide hormone (34.5 kDa), can be absorbed from the uterus into the systemic blood circulation in a biologically active form, and to compare the biological effects of continuous transendometrial (TE) administration of EPO with those of the pulsatile mode. Sprague-Dawley rats with gentamicin-induced anemia were treated with recombinant human erythropoietin (r-HuEPO) 200 U/kg per day for 5 days as follows: (1) the daily dose was instilled as a bolus through an indwelling cannula in the uterus; (2) the daily dose was continuously delivered into the uterus at a constant rate of 2 U/h by mini-osmotic pump connected to a similar cannula; (3) the third group received the daily r-HuEPO dose through the jugular vein; and (4) a control group treated with normal saline solution instilled in the uterus (as in group 1). The hematological parameters were measured for 53 days following initiation of r-HuEPO treatment. It was found that the biological effects following bolus daily transendometrial (TE) administration of EPO, expressed by red blood cell count and hematocrit levels, were equipotent to i.v. injection. On the other hand, the biological response following continuous TE EPO administration was significantly better than that observed following pulsatile administration. It is concluded that r-HuEPO is absorbed from the uterus in its bioactive form, with no deleterious effects on the uterine tissue. Slow release of EPO from a TE device may induce a beneficial biological response in comparison to pulsatile delivery.

Keywords: Erythropoietin; Intrauterine administration; Peptide; Absorption; Drug administration; Transendometrium; Controlled release; Pharmacodynamics

1. Introduction

A major drawback of peptide and protein drug therapy is their poor absorption through biological membranes (epithelia) and they are therefore generally administered by injection. Considerable research has been undertaken to develop methods that would make it possible to enhance peptidic drug transport across membranes (Banga and Chien, 1988). However, it has met with only very limited success (Borchardt et al., 1989).

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We recently described the absorption and systemic biological effect of two peptides: insulin (6.4 kDa) and calcitonin (3.45 kDa), following transendomeritrial (TE) administration in rats (Golomb et al., 1993). We discovered that both peptides are absorbed in biologically active form. The magnitude and duration of the hypoglycemic and the hypocalcemic effects induced by TE delivery of insulin and calcitonin, respectively, were equivalent to those obtained after subcutaneous injections, in both intact and ovariectomized rats. The major goal of the present investigation was to assess whether this new route of peptide administration for systemic effect is suitable for a much larger peptide such as EPO (34.5 kDa). This sialoglycoprotein hormone is mainly produced in the kidney and it regulates the production of red blood cells in mammals. It is used clinically to reverse anemia in patients with end-stage renal disease. Baldamus et al. (1989) administered the drug to rats with experimentally induced anemia and monitored the changes in hematological parameters.

Nagano et al. (1990) showed that the anemic state caused by the nephrotoxic effect of gentamicin is primarily due to a marked deficiency of endogenous EPO levels. This induced-anemia was controlled by daily intravenous bolus injections of recombinant human erythropoietin (r-HuEPO) 200 U/kg administered for 5 consecutive days. In this investigation we examined the effect following TE administration of r-HuEPO in the same model, in comparison to the same intravenous dose and regimen.

Administration of EPO for a long time period via the TE route can be achieved by incorporation of r-HuEPO in an intrauterine device (IUD) (Hoffman et al., 1993). Such a medicated IUD can be designed to provide the peptide in a slow release form. Therefore, it is important to compare the biological activity following pulsatile (once a day) vs continuous mode of EPO intrauterine administration. To assess the local side effects of prolonged r-HuEPO administration into the uterus, the endometrial tissues were examined histopathologically.

2. Materials and methods

2.1. Pharmacodynamic evaluation

Experimentally induced anemia was produced in female Sprague-Dawley rats (SD/HSD, Indianapolis, U.S.A.), weighing 200–240 g, by subcutaneous injection of gentamicin sulphate (100 mg/kg per day) (Cidomycin®, Roussel of Ireland, Dublin, Ireland) for 17 days, according to the procedure of Nagano et al. (1990). The anemic rats were randomly divided into four groups and three groups were treated with r-HuEPO (EPREX®, Cilag, Zug, Switzerland) as follows: (1) a bolus dose of 200 U/kg per day for 5 days administered through an indwelling polyethylene (PE 10) cannula inserted into the uterus through the vagina (leaving the uterus intact) (Golomb et al., 1993). (2) The same daily dose was continuously administered over 5 days through a similar cannula connected to an Alzet mini-osmotic pump (Model 2002 Alza Corp., Palo Alto, CA). The mini-osmotic pump was implanted subdermally in the abdominal area and was removed after 5 days. (3) The third group received the daily r-HuEPO dose as an intravenous bolus through a jugular vein cannula (Weeks and Davis, 1964), and the fourth group underwent the same procedure as group 1 but received only an equivalent volume of normal saline solution (controls). Blood samples (0.3 ml) were collected from the tail artery before gentamicin treatment (baseline values), and at days: 0 (the 18th day after commencement of the gentamicin administration, and just before initiation of r-HuEPO treatment), 3, 7, 10, 14, 26, 44, and 53. The hematological parameters were measured by means of a Coulter counter (Model S-plus Coulter Electronics, Luton, U.K.).

2.2. Histopathological examination

To assess the effect of continuous administration of r-HuEPO solution for 5 days on the uterine tissues, the uterus of healthy rats treated with r-HuEPO according to the same procedure

as group 1 were dissected and examined histopathologically. The uteri were fixed in 4% formaldehyde solution of phosphate buffer, pH 7.4. Three samples from each horn were trimmed for histology (one from the anterior segment, the second from the middle and the third from the distal segment). The segments were embedded in paraffin wax, sectioned at 4–5 microns thickness, stained by hematoxylin and eosin (HE), and examined using light microscopy. All slides were checked in a 'blind slide reading method' (i.e., the pathologist, A.N., did not have any knowledge of the slides' identity).

2.3. Statistical analysis

Student's t-test was used to evaluate the statistical significance of differences between each of the hematological parameters before and after gentamicin treatment. The significance of difference between the groups' hematological parameters at each time point was assessed by the nonparametric Kruskal Wallis test, followed by a Mann-Whitney test. Results are reported as mean ± SE.

3. Results

Daily administration of gentamicin for 17 days induced a moderate state of anemia in the rats, as indicated by the reduction in red blood cell (RBC) count as well as hemoglobin (HGB) and hematocrit (HCT) values (Table 1). This treatment was also associated with significantly moderate hypothermia, 36.0 ± 0.1°C in comparison to the temperature of 37.3 ± 0.4°C before treatment ($p < 0.001$).

Table 1
Hematological indices of rats following 17 days of daily administration of gentamicin sulphate 100 mg/kg

Variable	Before treatment	After treatment
Red blood cells (10^{12}/l)	7.3±0.3	6.2±0.1 [a]
Hemoglobin (g/dl)	14.6±0.6	11.4±0.4 [a]
Hematocrit (%)	39.6±1.6	31.4±1.9 [a]

Results are reported as mean ± SE, $n = 20$.
[a] Significantly lower than from respective control group.

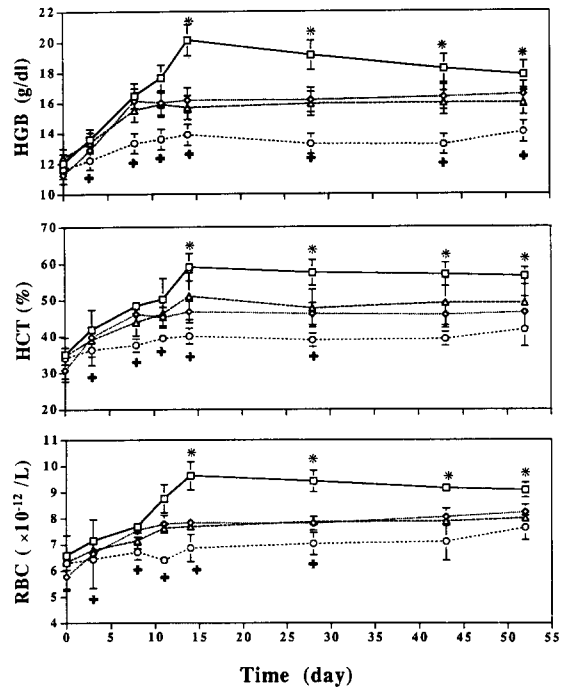

Fig. 1. The effect of r-HuEPO administration mode hematological parameters in anemic rats ($n = 5$). r-HuEPO was given for 5 days by: continuous intrauterine administration (□), a daily intravenous bolus dose (◇), a daily intrauterine bolus dose (△) and control, bolus administration of normal saline solution (○). HGB, hemoglobin content; HCT, hematocrit levels in (%); RBC, red blood cell count. The hematological values following continuous intrauterine administration were found to be significantly different from those of control group at all time points, and from those of bolus intravenous and intrauterine treatment modes (*). Significant difference between the bolus administrations and control values (+).

The effect of the various treatment modes with r-HuEPO for 5 days on relevant hematological indices is summarized in Fig 1. As can be seen, the biological effect of 200 U/kg per day r-HuEPO given as a daily bolus dose via either intravenous or TE administration was comparable at all time points. Both treatments caused a significant elevation of the RBC, HGB and HCT values in comparison to untreated rats. This elevation was evident 5 days after termination of the r-HuEPO treatments (day 10). Therapy by r-HuEPO dose in a sustained release fashion in-

duced the largest impact on the erythropoietic system. All of the hematological parameters measured (i.e., RBC, HGB, and HCT) were substantially greater than those obtained by the pulsatile mode of treatment (i.e., daily bolus). This was evident in all three parameters from day 10 on. The control group treated with saline solution regained normal hematological values at about day 15; however, all r-HuEPO-treated rats acquired statistically significant higher values of hematological parameters.

No pathological findings were noted in the treated animals in comparison to saline-treated and untreated animals following histopathologic examination (see Fig. 2).

4. Discussion

We have reported previously that polypeptide hormones such as calcitonin (3.45 kDa) and insulin (6 kDa), compounds with very poor absorbtion by nonparentral route, are equipotent to subcutaneous injections following intrauterine instillation. The present investigation shows that a much larger polypeptide – r-HuEPO with a molecular mass one order of magnitude greater (34 kDa) – is also absorbed via the TE route in its biologically active form. The peptide actually exerts an equipotent efficacy to intravenous administration as demonstrated by the elevation in RBC count and consequently elevation of both HGB and HCT values.

In an attempt to develop a non-parenteral route of administration of r-HuEPO for anemic patients, Mizuno et al. (1992) assessed the rectal absorption of r-HuEPO in rats. However, the drug was absorbed only with a promotor, and absolute bioavailability of only 1.2% was found. Although r-HuEPO blood concentrations were not measured in the present investigation, the availability of the drug via the TE route seems to be much better than through the rectal route, and close to that via the i.v. route. Moreover, as demonstrated in our previous work (Golomb et al., 1993), there is no need for a promotor for good bioavailability.

An intriguing outcome of this investigation is the fact that administration of the drug at a constant rate produces considerably greater biological effect than that observed in the pulsatile mode. This finding is in accord with previous observations in humans demonstrating that, despite the low bioavailability of the s.c. route (20–40%), s.c. injection of r-HuEPO is more effective than an i.v. injection (Bommer et al., 1987). In contrast to the steep decrease in serum levels after intravenous injection, EPO serum levels continued to be elevated for more than 2 days after s.c. r-HuEPO injection (Macdougall et al., 1989; Spivak, 1991). It seems that the excess hormone following bolus i.v. administration is

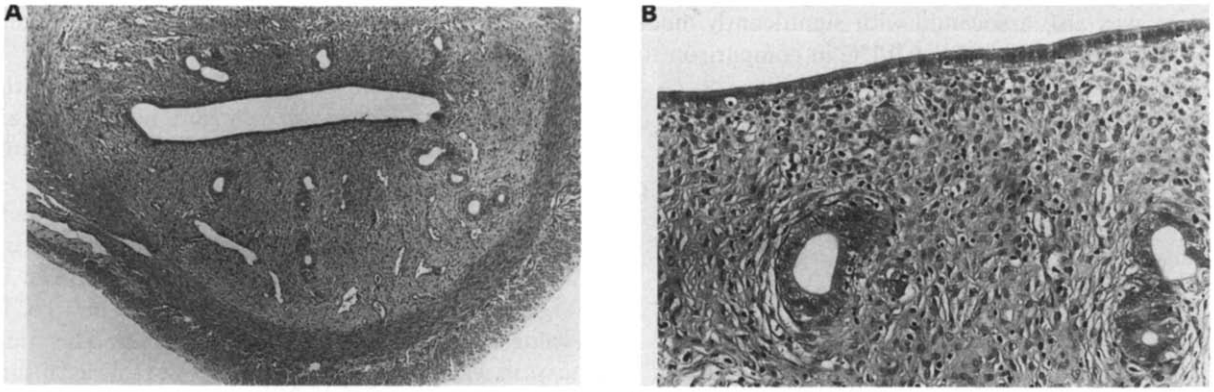

Fig. 2. Light micrograph showing normal appearance of cross-section of a uterine horn after 5 days of continuous r-HuEPO administration (magnification: A, ×50; B, ×250).

eliminated without having any biologic effect. The outcome of the present investigation is in accordance with these clinical reports supporting the hypothesis that the continuous availability of intermediate plasma concentrations rather than peak concentrations is essential for greater efficacy of r-HuEPO treatment. These researchers indicate that bioavailability alone is an inappropriate parameter for predicting r-HuEPO effectiveness (Macdougall et al., 1989; Spivak, 1991). It could be suggested that the long-term availability of low EPO concentrations actually mimics the physiological situation (Spivak, 1991) and does not lead to saturation of tissue receptors, unlike the situation following bolus administration.

Administration of EPO, like many other large polypeptide hormones, can only be accomplished by parenteral administration. Since in most cases these treatments are chronic in nature and sometimes for lifetime, this route of administration is associated with considerable discomfort and danger to the patient. The TE route is a new route of polypeptide administration for systemic effect. This route is particularly suitable for long-term controlled release of drugs, since the controlled release device similar to an IUD can be implanted in the cavity of the uterine lumen. The amount of hormone that is required for a year of treatment is in the order of milligrams which could be easily incorporated in a device smaller than contemporary IUDs. To the best of our knowledge, we are the first to report on the systemic therapeutic effect of drugs following TE delivery. The use of medicated IUD for long-term, continued intrauterine administration of contraceptive steroids and copper for local, site-specific effect is well known (Chi, 1993; I-cheng, 1993; Rybo et al., 1993). An intrauterine controlled release system could provide a constant rate of drug release available for TE absorption over a long period of time (Golomb et al., 1993). The outcome of this investigation provides a pharmacodynamic rationale for utilizing this pharmaceutical approach with EPO.

The histopathologic examination confirmed that the uterus remained intact following r-HuEPO administration. This finding proves that the drug could not have been absorbed via an injured region in the uterus. The histopathologic results are in agreement with previous findings where 14 days of TE calcitonin administration resulted in no local side effects (Golomb et al., 1993; unpublished data). The severe drawbacks of EPO parenteral treatment and the fact that EPO is contraindicated during pregnancy (McEvoy, 1993) make the TE route of administration suitable in both pre- and postmenopausal women. An IUD containing EPO could serve a double purpose: to treat anemia and to act as a contraceptive device. However, several important issues associated with the use of IUD for contraception should be addressed in the development of a TE drug delivery system.

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