Inhibition of Prolyl Hydroxylases Increases Erythropoietin Production in ESRD

Wanja M. Bernhardt,* Michael S. Wiesener,*† Paul Scigalla,‡ James Chou,§ Roland E. Schmieder,* Volkmar Günzler,§ and Kai-Uwe Eckardt*

*Department of Nephrology and Hypertension and †Interdisciplinary Centre for Clinical Research, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany; ‡International Clinical Research Consulting, Berlin, Germany; and §FibroGen Inc., South San Francisco, California

ABSTRACT

The reasons for inadequate production of erythropoietin (EPO) in patients with ESRD are poorly understood. A better understanding of EPO regulation, namely oxygen-dependent hydroxylation of the hypoxia-inducible transcription factor (HIF), may enable targeted pharmacological intervention. Here, we tested the ability of fibrotic kidneys and extrarenal tissues to produce EPO. In this phase 1 study, we used an orally active prolyl-hydroxylase inhibitor, FG-2216, to stabilize HIF independent of oxygen availability in 12 hemodialysis (HD) patients, six of whom were anephric, and in six healthy volunteers. FG-2216 increased plasma EPO levels 30.8-fold in HD patients with kidneys, 14.5-fold in anephric HD patients, and 12.7-fold in healthy volunteers. These data demonstrate that pharmacologic manipulation of the HIF system can stimulate endogenous EPO production. Furthermore, the data indicate that deranged oxygen sensing—not a loss of EPO production capacity—causes renal anemia.


In adults, the kidneys are the main site of production of the glycoprotein hormone erythropoietin (EPO), which links renal function to erythropoiesis. EPO production is normally inversely related to blood oxygen content, thus establishing an efficient feedback control of red cell production.1 In the presence of chronic kidney disease (CKD), plasma EPO concentrations fail to increase with declining hemoglobin (Hb) levels, causing the development of renal anemia. Peritubular cortical fibroblasts in the kidney have been identified as the sites of EPO synthesis, but why the kidneys lose their ability to produce sufficient EPO in progressive renal disease remains unclear.2,3

EPO is part of a widespread system of hypoxia-inducible gene expression mediated by hypoxia-inducible transcription factors (HIFs).4,5 HIFs are composed of one of two oxygen-regulated α-subunits (HIF-1α and HIF-2α) that form heterodimers with a constitutive HIF-β subunit. HIF-2α is the HIF isoform responsible for regulation of EPO.6–9 Stability and transcriptional activity of HIF-α is regulated by molecular oxygen: Oxygen-dependent hydroxylation of two prolyl residues leads to binding of an E3-ubiquitin–ligase complex that targets HIF for rapid proteasomal degradation.10,11 Hydroxylation of an asparagyl residue interferes with binding of the transcriptional co-activator CBP/p300. These hydroxylation reactions are mediated by a family of prolyl-hydroxylase domain (PHD) enzymes and a related asparagyl-hydroxylase, which require oxygen and oxoglutarate as substrates. Oxoglutarate analogues can therefore func-
tion as competitive prolyl-hydroxylase inhibitors (PHD-I). Compounds of this class have been shown to induce HIF-target genes in preclinical in vitro and in vivo models. Here, we used FG-2216, an orally active small molecule PHD-I, in a single-dose phase I study of nephric and anephric patients who had ESRD and were on regular dialysis to test the ability of diseased kidneys and of extrarenal tissues to produce EPO.

RESULTS

Patient Characteristics
Baseline data of the healthy control subjects and two groups of hemodialysis (HD) patients who were enrolled in the study are shown in Table 1. The reasons for bilateral nephrectomy included chronic urinary tract infection, polycystic kidney disease, refractory hypertension, and bilateral renal carcinoma. The mean weekly epoetin dosage before the study was 9167 mU in nephric HD patients and approximately 7333 mU in anephric HD patients (assuming that 1 μg of darbepoetin is equivalent to 200 mU epoetin).

Pharmacodynamic Effects/Plasma EPO Levels
In all participants, FG-2216 significantly increased plasma EPO concentrations. In control subjects, median plasma EPO rose from 6.4 U/L at baseline to a maximum of 81.2 U/L at 12 hours after the dose (Figure 1A). Nephric HD patients showed the largest rise in plasma EPO, with median levels increasing from 7.8 U/L at baseline to 240.6 U/L at 24 hours (Figure 1B). Although the median plasma EPO concentration peaked at 24 hours, plasma EPO of three patients in this group already decreased between 12 and 24 hours. One anephric patient (patient 4) was accidentally underdosed, receiving 250 mg (approximately 4 mg/kg) rather than 1250 mg of FG-2216. When this patient was excluded from the summary analysis, the median plasma EPO concentrations increased from 4.4 to 63.1 U/L in anephric patients (Figure 1C).

Reticulocytes tended to decrease in HD patients after withdrawal from recombinant human EPO (rhEPO). From dosing until day 7 of the study, the reticulocyte count increased significantly in nephric HD patients, the group with the highest median plasma EPO levels (from 39.37 ± 13.56 to 52.73 ± 27.8/μl; P < 0.05), and they did not decrease further in the anephric HD patients. In all groups, there were no significant changes in Hb levels during the study period (Table 2).

Pharmacokinetics of FG-2216
The pharmacokinetic data of FG-2216 are summarized in Table 3. The urinary excretion of unchanged FG-2216 in healthy control subjects amounted to 11.0 ± 3.5% of the administered dose within 48 hours. Compared with healthy control subjects, HD patients had comparable FG-2216 T_max and C_max levels but lower apparent clearance (Cl/F), longer half-life (T 1/2) values, and a higher area under the curve (AUC; Table 3).

No change of FG-2216 plasma levels was observed during the dialysis period, and the dialysis procedure removed only a small fraction of the administered FG-2216 dose (1.01 ± 0.53 and 1.77 ± 0.45% in nephric and anephric HD patients, respectively). The patient who received only one fifth of the intended dose (patient 4 in the anephric group) had a lower C_max and AUC, but all other pharmacokinetic parameters were within the range of all HD patients. All HD patients except one had anuria during the collection period; the patient with residual urine production excreted 2% of the administered dose (27 mg) in 1900 ml urine in 48 hours.

Safety
There was no clinically significant change in laboratory and clinical findings. In total, 17 adverse events were recorded: Two in control subjects, six in nephric HD patients, and nine in anephric HD patients. These included diarrhea, abdominal discomfort, nausea, lesion of a radial nerve branch caused by venipuncture, rash, headache, hyperkalemia, dizziness, sweating, arterial hypotension, an accidental scalp wound. Two serious adverse events occurred, both of which were considered not to be drug related. One nephric HD patient required an additional HD session because of hyperkalemia attributed to dysfunction of his arteriovenous fistula. One anephric patient was hospitalized after a transplant offer, but transplantation could not be performed for donor reasons.

During the study period, repetitive blood samples were drawn for clinical chemistry (day −7, day 0, day 1, day 3 or 4, and day 7). Overall, we did not observe a significant change in all parameters tested, including iron parameters, liver function tests, and lipase levels, in all groups after administration of FG-2216. Values at baseline (day −7) and at day 7 are shown in Table 2.

DISCUSSION
This proof-of-concept study provides unexpected novel insights into the pathogenesis of renal anemia. Unused production capacity for EPO, presumably as a result of desensitization of the oxygen-sensing mechanism rather than destruction of its cellular production sites, seems to cause the inappropriately low production of the hormone in patients with CKD.

Previous observations showed that plasma EPO concentrations in dialysis patients can increase in response to acute episodes of hypoxia, despite not responding adequately to the chronic reduction in Hb levels. Moderate inverse responses of plasma EPO to changes in Hb levels induced by phlebotomy and transfusion have also been reported. Moreover, it was recently found that with increasing altitude, HD patients receive lower doses of rhEPO and yet achieve higher hematocrit levels, which could also indicate residual hypoxia-stimulated EPO production. The source of EPO under all of these circumstances, however, remains unclear. Our comparative analysis of nephric and anephric HD patients clearly indicates that both the diseased kidneys and extra-renal sites can contribute to a marked rise in plasma EPO concen-
<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Body Weight (kg)</th>
<th>Time on Dialysis (months)</th>
<th>Cause of ESRD or Time and Reason for Nephrectomy</th>
<th>Regular rhEPO Treatment (Mean Weekly Dosage)</th>
<th>Comorbidity</th>
<th>Diabetes</th>
<th>Hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>43</td>
<td>115</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>37</td>
<td>88</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>44</td>
<td>82</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>36</td>
<td>94</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>47</td>
<td>73</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>37</td>
<td>100</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>HD patients with native kidneys in situ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>37</td>
<td>68</td>
<td>20</td>
<td>Tubulointerstitial nephritis, chronic hypokalemia and anorexia</td>
<td>18,000 IU Epoetin-β intravenously</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>35</td>
<td>115</td>
<td>38</td>
<td>Membranoproliferative glomerulonephritis</td>
<td>9000 IU Epoetin-β subcutaneously</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>43</td>
<td>72</td>
<td>114*</td>
<td>Susception of Alport syndrome</td>
<td>15,000 IU Epoetin-β intravenously</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>46</td>
<td>83</td>
<td>98</td>
<td>Interstitial nephritis and nephrosclerosis</td>
<td>2000 IU Epoetin-α intravenously</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>34</td>
<td>76</td>
<td>47</td>
<td>Nephrosclerosis</td>
<td>9000 IU Epoetin-β intravenously</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>39</td>
<td>84</td>
<td>32</td>
<td>Chronic glomerulonephritis</td>
<td>2000 IU Epoetin-α intravenously</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>HD patients with bilateral nephrectomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>27</td>
<td>65</td>
<td>87</td>
<td>Chronic pyelonephritis (r: 8/2000; l: 10/2001)</td>
<td>7000 IU Epoetin-β subcutaneously</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>61</td>
<td>61</td>
<td>99</td>
<td>ADPKD (?; 1998 preparation for KTx; l: 9/2004 cyst bleeding)</td>
<td>40 µg Darbepoetin alfa intravenously</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>51</td>
<td>59</td>
<td>71</td>
<td>Refractory, severe hypertension (bl: 2001)</td>
<td>9000 IU Epoetin-β intravenously</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>51</td>
<td>68</td>
<td>101</td>
<td>Recurrent UTI, medullary sponge kidneys (bl: 1977)</td>
<td>6000 IU Epoetin-β subcutaneously</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>82</td>
<td>60</td>
<td>64</td>
<td>Bilateral RCC (bl: 12/2001)</td>
<td>4000 IU Epoetin-β intravenously</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

ADPKD, autosomal dominant polycystic kidney disease; bl, bilateral nephrectomy; KTx, kidney transplantation; l, left nephrectomy; r, right nephrectomy; RCC, renal cell carcinoma; UTI, urinary tract infection.

*Cumulative time on HD interrupted by episodes of KTx, no renal allograft in situ at the time of study participation.
With pharmacologic activation of HIF. Besides the kidneys, the liver is a site of relevant EPO production, producing most EPO during fetal life and contributing up to one third to total EPO production in animals who are exposed to severe hypoxia. EPO production in anephric patients is therefore highly likely to be of hepatic origin, although it is obviously impossible to prove this assumption.

A particularly striking feature of our observations is the magnitude of the rise in plasma EPO evoked by FG-2216. The increase in anephric individuals was similar to that in healthy volunteers, although one might have expected a fraction of the normal response, corresponding to the hepatic contribution to EPO formation. In HD patients with their diseased kidneys in situ, the rise in plasma EPO was approximately fourfold higher than in normal individuals. The systemic exposure to FG-2216 was significantly enhanced in patients with kidney failure, with a 1.65-fold increase in the AUC of its plasma concentrations; however, this increase was primarily due to an increase in plasma T1/2. T_max and C_max were similar in dialysis patients and healthy individuals, so FG-2216 exposure in the first hours after dosing was similar in both groups. Nevertheless, plasma EPO concentrations in nephric HD patients tended to be higher and in anephric HD patients were similar to those in control subjects after 4 hours. Therefore, the increased response cannot be explained on the basis of pharmacokinetics only; rather, it may indicate an increased sensitivity to the drug, the molecular basis of which remains to be elucidated.

Despite significant progress in understanding the oxygen-sensing mechanisms that regulate HIF stability and transcriptional activity, the overall control of EPO production in the kidney and its relationship to tissue oxygen tensions, oxygen supply, and oxygen consumption by tubular cells remain incompletely understood. Observations in the early posttransplantation period indicate that excretory kidney function and the ability to produce EPO may dissociate. Tubular function is probably a prerequisite for the adaptation of EPO formation to changes in blood oxygen content. In fact, it is possible that, as a result of the lack of tubular oxygen consumption, renal oxygen tensions in diseased kidneys are higher than in intact kidneys; however, other evidence points toward an important role of hypoxia in the progression of kidney disease.

Irrespective of the mechanisms that impair the normal feedback control of erythropoiesis in patients with CKD, stimulation of endogenous EPO production through HIF stabilization offers a potential novel opportunity for the treatment of anemia, and our results indicate that this approach could potentially be extended to patients who are on dialysis. The magnitude of the increase in plasma EPO observed in this study for nephric HD patients is comparable to plasma EPO concentrations achieved after subcutaneous administration of rhEPO, using dosages that are typically administered for anemia correction. Moreover, the pharmacokinetic data obtained here indicate that accumulation of FG-2216 can be avoided if dosing intervals are adjusted according to its prolonged plasma T1/2 in kidney failure.

In summary, this study provides proof of principle that pharmacologic inhibition of HIF PHD enzymes with an oral agent can induce endogenous EPO in dialysis patients; however, the safety of any therapeutic long-term activation of the HIF system requires careful consideration, given the broad biological potential of HIF in mediating hypoxia-driven processes. Interestingly, genetic causes of impaired degradation of HIF,
Anephric HD patients 4.0
Nephric HD patients 3.0

Transferrin saturation (%; 16 to 45) 30.7

liver parameters, Hb, iron parameters, or lipid profile. Data are means

Study Protocol

Patients

CONCISE METHODS

Patients

Twelve long-term HD patients and six healthy control subjects were

potentially comparable to chronic pharmacologic inhibition of

HIF degradation, have been identified as causes of rare polycy-

themias.32,33

Concise Methods

Patients

Twelve long-term HD patients and six healthy control subjects were

enrolled after informed consent. Six HD patients were anephric, and six

had their native kidneys in situ; none carried a renal transplant. Major

exclusion criteria were a history of malignancy within 5 years, recent

thromboembolic events, evidence of active infection or inflammatory
disease, active bleeding, and severe congestive heart failure.

Study Protocol

The study was an open-label, single-dose phase I study to investi-
gate the pharmacokinetics, biological activity, and safety of the novel,

orally active PHD-1 FG-2216 (FibroGen Inc., South San Francisco,

CA; Eudra cat. no. 2005-003664-29). In all HD patients, treatment

with rhEPO was paused 7 days before study initiation. All participants

were to receive a single dose of 20 mg/kg body wt FG-2216 on day 1

and were followed up for 7 days. In HD patients, FG-2216 was admin-

istered on the morning after their first HD of the week. Pharmacokinet-

ic plasma levels of FG-2216 were determined before dosing and

between 0.5 and 24 hours (all groups) and after 48 hours (control

subjects) or 72 hours (HD patients) using mass spectroscopy. The
dialyzer clearance of FG-2216 was determined in HD patients at day 2
(24 hours after dosing), using a standardized HD protocol: Low-flux
dialyzer (Hemoflow F6HPS; Fresenius Medical Care, Bad Homburg,
Germany), 4-hour dialysis time, blood flow of 200 to 250 ml/min, and
dialysate flow of 500 ml/min. EPO concentrations were determined

before dosing and 4, 8, 12, and 24 hours after dosing, using a com-
mercially available ELISA-kit (R&D Systems).

Statistical and Analysis

All results are expressed as mean ± SD with the exception of

Plasma EPO levels, which are expressed as median. Descriptive statis-
tical analyses were performed using t test and Mann-Whitney U test

for pharmacokinetic data and reticulocyte count, using SPSS 15.0 for

Windows. P < 0.05 was considered significant.

Acknowledgments

The expert support of U. Heinritz and other staff members of the

clinical research unit at the Department of Nephrology and Hyper-
tension at FAU is gratefully acknowledged. Drs. B. Bueschges-
Seraphin and J. Nikolay (Fürth), J. Wopperer (Neumarkt), J. Mann
(Munich), and D. Soreth-Rieke (Miesbach) provided invaluable sup-
port in recruiting the patients and cooperated in the study perfor-
mance. Gert Kochendoerfer from FibroGen helped to develop the

study protocol.

Disclosures

P.S. was working as a consultant for FibroGen, and V.G. and J.C. are em-
ployees of FibroGen.

References

1. Jelkmann W: Erythropoetin: Structure, control of production, and


<table>
<thead>
<tr>
<th>Laboratory Parameter (Normal Range)</th>
<th>Baseline (Day −7)</th>
<th>End of Study (Day 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Subjects</td>
<td>Nephric HD Patients</td>
</tr>
<tr>
<td>AST (IU/L; &lt; 31)</td>
<td>24.5 ± 9.5</td>
<td>14.8 ± 4.8</td>
</tr>
<tr>
<td>ALT (IU/L; &lt; 34)</td>
<td>35.5 ± 20.9</td>
<td>13.7 ± 8.8</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl; 0.1 to 1.1)</td>
<td>0.65 ± 0.32</td>
<td>0.57 ± 0.16</td>
</tr>
<tr>
<td>Albumin (g/L; 35 to 55)</td>
<td>41.7 ± 1.6</td>
<td>42.0 ± 1.3</td>
</tr>
<tr>
<td>AP (U/L; 42 to 98)</td>
<td>76.7 ± 19.2</td>
<td>99.8 ± 14.3</td>
</tr>
<tr>
<td>Lipase (U/L; &lt; 60)</td>
<td>21.2 ± 17.5</td>
<td>56.7 ± 25.2</td>
</tr>
<tr>
<td>Hb (g/dl; 12 to 16)</td>
<td>15.40 ± 0.70</td>
<td>13.30 ± 1.60</td>
</tr>
<tr>
<td>Ferritin (22 to 112)</td>
<td>168 ± 124</td>
<td>1108 ± 496</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>30.7 ± 13.1</td>
<td>35.1 ± 11.6</td>
</tr>
</tbody>
</table>

When baseline levels at screening visit were compared with measurements 1 week after study drug administration, FG-2216 caused no significant changes in

liver parameters, Hb, iron parameters, or lipid profile. Data are means ± SD (n = 6 per group). AST, aspartate transaminase; ALT, alanine transaminase; AP, alkaline phosphatase.

Table 2. Blood chemistry before (day −7, baseline visit) and 1 week after (day 7, final study visit) administration of FG-2216

<table>
<thead>
<tr>
<th>Group</th>
<th>T (_{max}) (hours)</th>
<th>C (_{max}) (µg/ml)</th>
<th>AUC (hr/µg per ml)</th>
<th>T (_{1/2}) (hours)</th>
<th>Clearance (ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td>3.5 ± 1.8</td>
<td>179.0 ± 31.0</td>
<td>3660 ± 813</td>
<td>15.7 ± 1.1</td>
<td>530 ± 172</td>
</tr>
<tr>
<td>Nephric HD patients</td>
<td>3.0 ± 1.1</td>
<td>156.0 ± 17.1</td>
<td>5730 ± 2420</td>
<td>29.8 ± 11.4</td>
<td>311 ± 125</td>
</tr>
<tr>
<td>Anephric HD patients</td>
<td>4.0 ± 2.5</td>
<td>159.0 ± 18.8</td>
<td>6572 ± 2349</td>
<td>33.3 ± 8.4</td>
<td>208 ± 91</td>
</tr>
</tbody>
</table>

Data are means ± SD. The AUC is significantly increased and the plasma T \(_{1/2}\) is twice as long in HD patients in comparison with healthy control subjects (P = 0.015 for nephric HD and P = 0.004 for anephric HD versus control subjects but not different between nephric and anephric HD patients).

Table 3. Pharmacokinetics of FG-2216. T \(_{max}\) and C \(_{max}\) are not different among groups


