Supporting Information

Brines *et al.* 10.1073/pnas.0805594105

SI Materials and Methods

Hematopoietic Potency. Peptides were tested *in vitro* for hematopoietic potency by use of the EPO-responsive human erythroleukemic cell line UT-7 EPO as previously described in detail (1, 2). The assay was performed over 48 h, and proliferation was quantified by using WST-1 reduction (Roche; no. 1644807). As shown in supporting information (SI) Fig. S1, HBP over the range of 5 pM to 50 nM did not increase cell number, in contrast to the large hematopoietic effect of EPO.

Additional experiments were performed in which the rat or rabbit received repeated injections of pHBSP. Specifically, pHBSP was administered twice daily i.v. to Sprague–Dawley rats for 28 days. For this study, nine male and nine female rats were assigned to Groups 1–4 receiving 0, 60, 180, and 600 μg/kg per dose (0, 48, 143, and 477 nmol/kg, respectively) of pHBSP in PBS by bolus i.v. administration from days 1–28. Blood samples to assay for hematological variables were collected on day 29. There was no difference between any of the groups in hemoglobin concentration (Fig. S2).

Further, pHBSP was administered twice daily i.v. to New Zealand White rabbits for 28 days. For this study, six males and six females were assigned to Groups 1 and 4, and four male and four female rabbits were assigned to Groups 2 and 3 receiving 0 (Group 1), 30 (24 nmol/kg; Group 2), 90 (72 nmol/kg; Group 3), and 300 (240 nmol/kg; Group 4) μg/kg per dose pHBSP in PBS by bolus i.v. administration. Comparison of baseline versus day 29 hematological parameters showed no difference in hemoglobin concentration, hematocrit, or platelet count (Figs. S3–S5). Thus, studies carried out in two species confirm that pHBSP is not erythropoietic *in vivo*.

Pharmacokinetics of pHBSP. ***Rat.*** The pharmacokinetic behavior of pHBSP was determined after a single i.v. dose to male Sprague–Dawley rats ported with bilateral jugular vein cannulae. Two groups, each of three rats, received either 60 μg/kg (48 nmol/kg; Group 1) or 180 μg/kg (143 nmol/kg; Group 2) of pHBSP diluted in PBS. The dose was administered over a period of 10–12 sec into one of two ports, and samples were withdrawn from the other port predose and at 2, 4, 6, 8, 10, 12, 14, 16, and 18 min after administration of the dose. Plasma concentrations of pHBSP were determined by using a liquid chromatography–mass spectroscopy assay.

Plasma pHBSP concentration as a function of time after dose is shown in Fig. S6. Individual and group mean pharmacokinetic parameters are shown in Table S1. The mean peak drug concentration (C_{max}) values were 254.67 ($\pm$53.59) and 1,103.67 ($\pm$194.53) ng/ml for Groups 1 and 2, respectively. Both C_{max} and area under the concentrations vs. time curve (area under the curve; AUC) increased with increasing dose in a slightly more than dose-proportional manner, although the variability between animals within each dose group was high. The mean $t_{1/2}$ was 0.028 h for Group 1 and 0.047 h for Group 2.

Rabbit. The pharmacokinetic behavior of pHBSP was determined after a single i.v. dose to male New Zealand White rabbits. Two groups, each with three rabbits, received either 30 μg/kg (24 nmol/kg; Group 1) or 90 μg/kg (72 nmol/kg; Group 2) of pHBSP diluted in PBS. The dosing solution was administered over a period of 10–12 sec via ear vein. Blood samples were collected from the contralateral ear by venipuncture of a central auricular artery or a marginal vein. Samples were withdrawn predose and at 2, 4, 6, 8, 10, 12, 14, 16, and 18 min postdose. Plasma pHBSP concentration as a function of time after dose is shown in Fig. S7. Individual and group mean pharmacokinetic parameters are shown in Table S2.

The mean C_{max} values were 95.10 ($\pm$44.34) and 200.67 ($\pm$52.92) ng/ml for Groups 1 and 2, respectively. Both C_{max} and AUC increased with increasing dose in a less than dose-proportional manner. Similar to the observations in rats, variability in measured plasma drug concentrations in rabbits was high with near overlap between C_{max} and AUC values between animals in the two dose groups. The mean $t_{1/2}$ was 0.028 h for Group 1 and 0.038 h for Group 2.

1. Erbayraktar S, *et al.* (2003) Asialoerythropoietin is a nonerythropoietic cytokine with broad neuroprotective activity *in vivo*. *Proc Natl Acad Sci USA* 100:6741–6746.

2. Leist M, *et al.* (2004) Derivatives of erythropoietin that are tissue protective but not erythropoietic. *Science* 305:239–242.

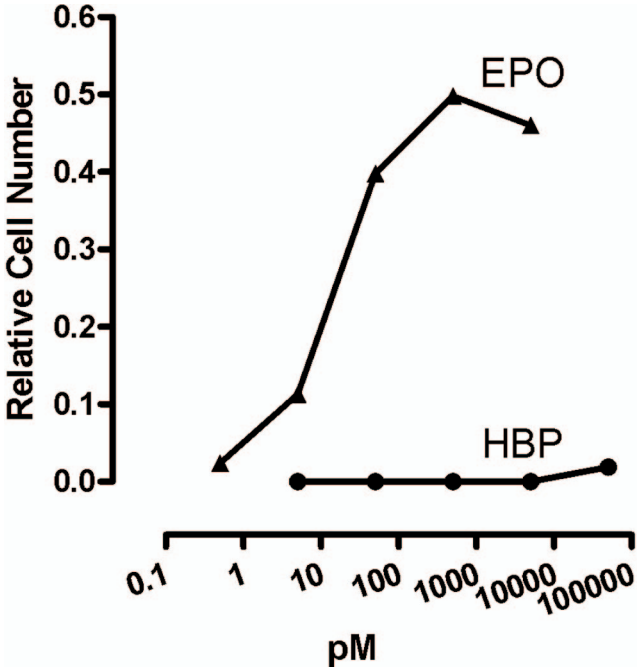

Fig. S1. Peptide HBP has no effect on cell number in the UT-7 EPO assay. In contrast, EPO promotes cell growth and is, therefore, hematopoietic.

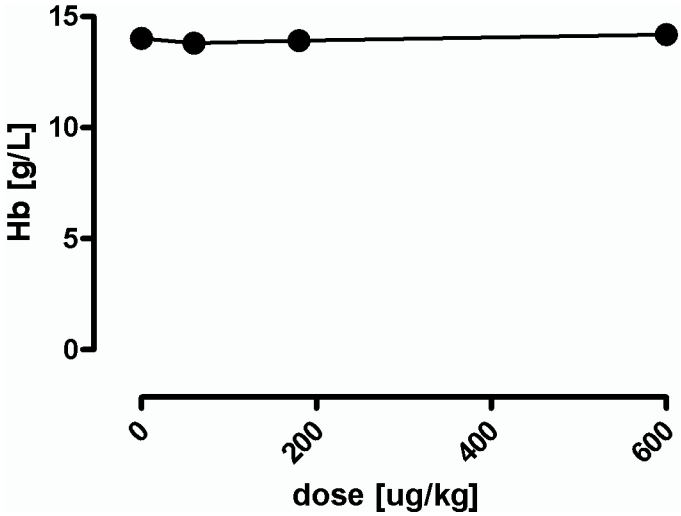

Fig. S2. pHBSP is not erythropoietic *in vivo*. Sprague–Dawley rats administered peptide twice daily i.v. at the indicated dosages did not exhibit changes in hemoglobin concentration over a 28-day period.

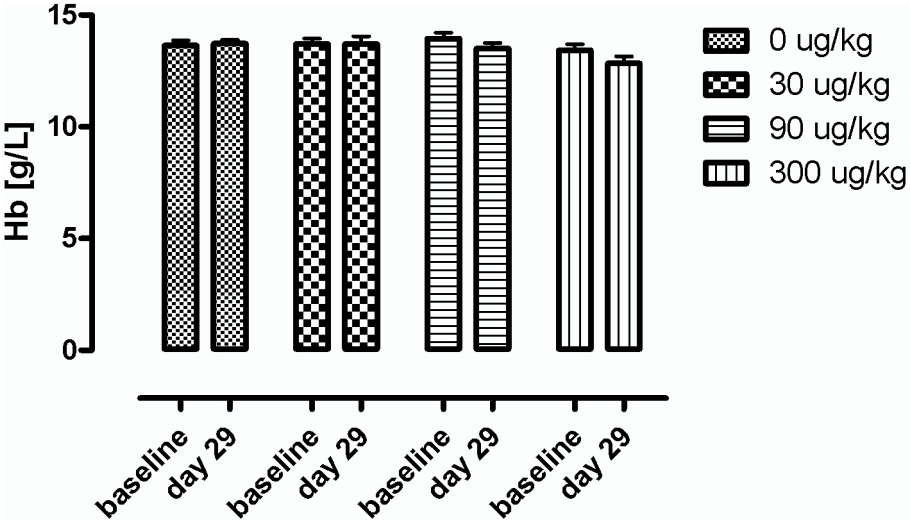

Fig. S3. Rabbits administered pHBSP twice daily i.v. did not exhibit changes in hemoglobin concentration over 28 days of administration.

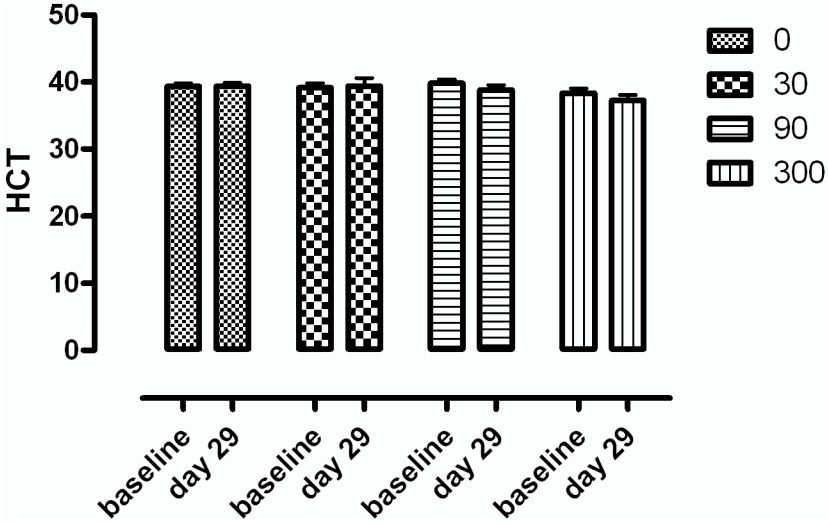

Fig. S4. No change in the hematocrit was observed in rabbits administered pHBSP twice daily for 28 days.

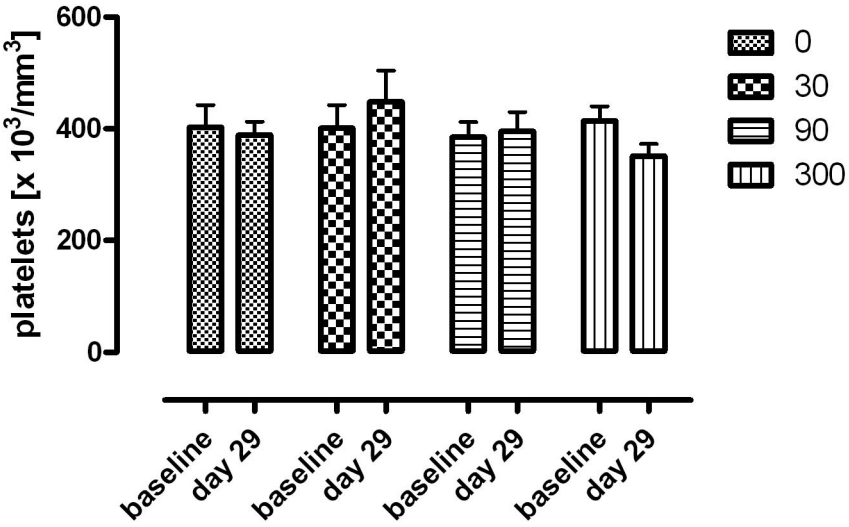

Fig. S5. Platelet count did not change over 28 days after the administration of pHBSP twice daily to rabbits.

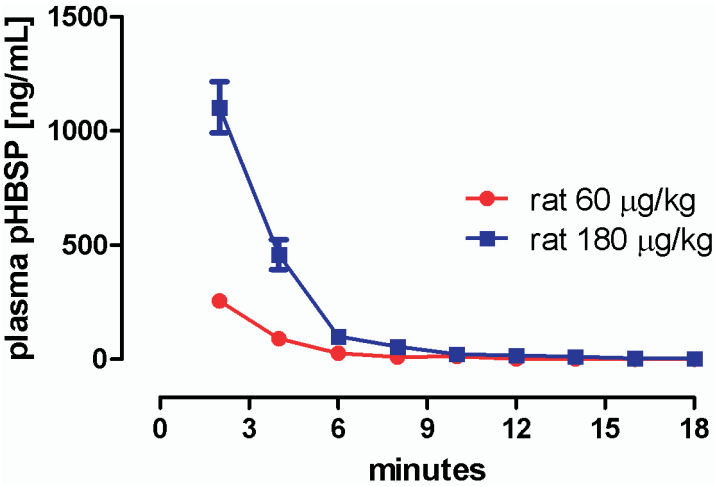

Fig. S6. Mean plasma levels of pHBSP in rats over time after a single dose at the indicated amount. The mean half-life in the rat was estimated to be ≈2 min.

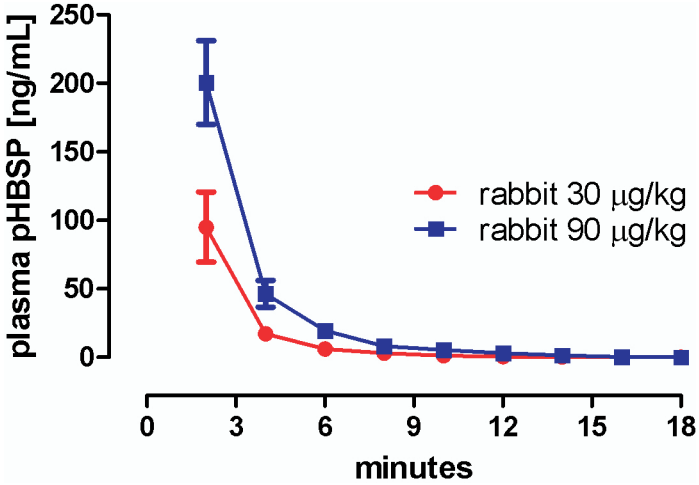

Fig. S7. Mean plasma levels in rabbits over time after a single dose of pHBSP at the indicated amount. The mean half-life in the rabbit was estimated to be ≈2 min.

Table S1. Pharmacokinetic parameters after a single i.v. bolus dose of pHBSP in rats

Subject	Body wt, kg	Dose, μg/kg	T_{max}, hr	C_{max}, ng/ml	AUC LAST, h·ng/ml	AUC inf, h·ng/ml	$t_{½}$, h
1	0.282	60.00	0.033	281.00	26.43	26.52	0.015
2	0.251	60.00	0.033	290.00	34.19	34.29	0.017
3	0.288	60.00	0.033	193.00	18.70	18.93	0.050
Mean	0.27	60.00	0.033	254.67	26.44	26.58	0.028
SD	0.02			53.59	7.74	7.68	0.020
4	0.283	180.00	0.033	911.00	80.10	80.99	0.063
5	0.294	180.00	0.033	1,300.00	134.08	134.44	0.045
6	0.318	180.00	0.033	1,100.00	96.37	96.75	0.034
Mean	0.30	180.00	0.033	1,103.67	103.52	104.06	0.047
SD	0.02			194.53	27.69	27.47	0.014

Nominal time and dosage used for pharmacokinetic analysis. Median is calculated for T_{max}., the time at which C_{max} (maximum observed plasma concentration) occurs. AUC LAST, area under the curve from time 0 to last measured concentration; AUC inf, area under the curve from time 0 to infinity.

Table S2. Pharmacokinetic parameters after a single i.v. bolus dose of pHBSP in rabbits

Subject	Body wt, kg	Dose, μg/kg	T_{max}, h	C_{max}, ng/ml	AUC LAST, h·ng/ml	AUC inf, h·ng/ml	$t_{½}$, h
1	3.51	30.00	0.033	126.00	16.33	16.50	0.027
2	3.00	30.00	0.033	115.00	16.36	16.52	0.029
3	3.07	30.00	0.033	44.30	4.72	*	*
Mean	3.19	30.00	0.033	95.10	12.47	16.51	0.028
SD	0.28			44.34	6.71	0.01	0.002
4	2.99	90.00	0.033	212.00	22.20	22.33	0.039
5	3.08	90.00	0.033	247.00	30.64	30.78	0.045
6	3.07	90.00	0.033	143.00	18.03	18.17	0.030
Mean	3.05	90.00	0.033	200.67	23.63	23.76	0.038
SD	0.05			52.92	6.43	6.42	0.008

Nominal time and dosage used for pharmacokinetic analysis. Median is calculated for T_{max}.
*Not enough data available to calculate given parameter.