Supplementary information

Contents

Validation of HBP ELISA kit for rat samples

Before measurement of HBP in rat samples, the kit was validated for use with rat plasma, urine, and organ lysates by a spike and recovery assay. Plasma, urine and organ homogenate samples from control rats were spiked with 1.25 ng/mL of recombinant HBP, a level near the middle of the detectable range of the kit. HBP level was measured in the spiked and un-spiked sample and the percent recovery of HBP was determined and shown in supplementary table S1. The background level in each sample type is shown in supplementary table S2.

Supplementary table S1. Recovery of spiked HBP in various samples, n=3 of each type, expressed as mean ± standard deviation.

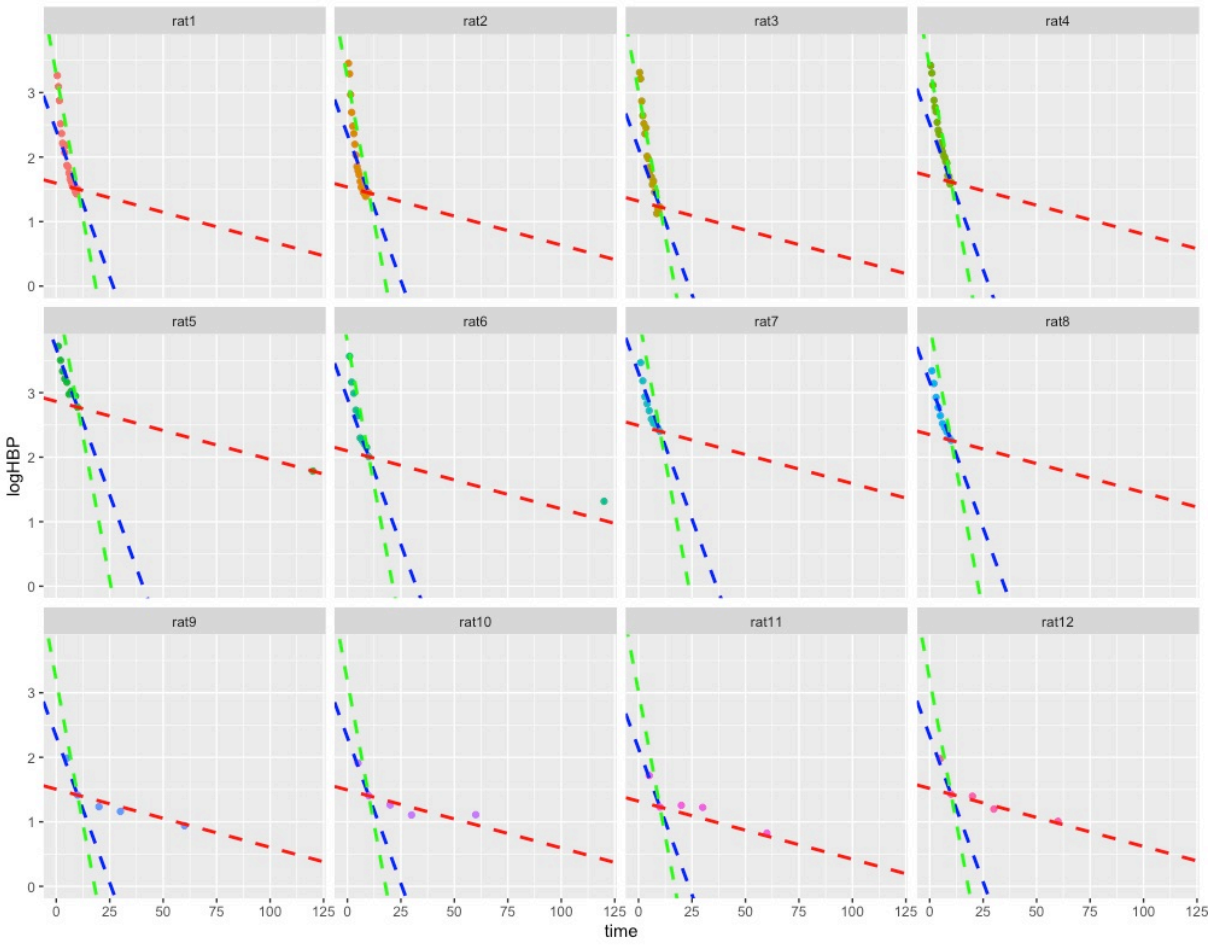
Sample	Recovery (1:40 dilution)	Recovery (1:400 dilution
plasma	90 ± 6 %	
liver	112 ± 28 %	106 ± 18%
spleen	104 ± 5%	
kidney	94 ± 9 %	
lung	102 ± 8%	
urine	100 ± 15%	

Supplementary table S2. Background level in various samples, n=3 of each type, expressed as mean ± standard deviation (ng/mL).

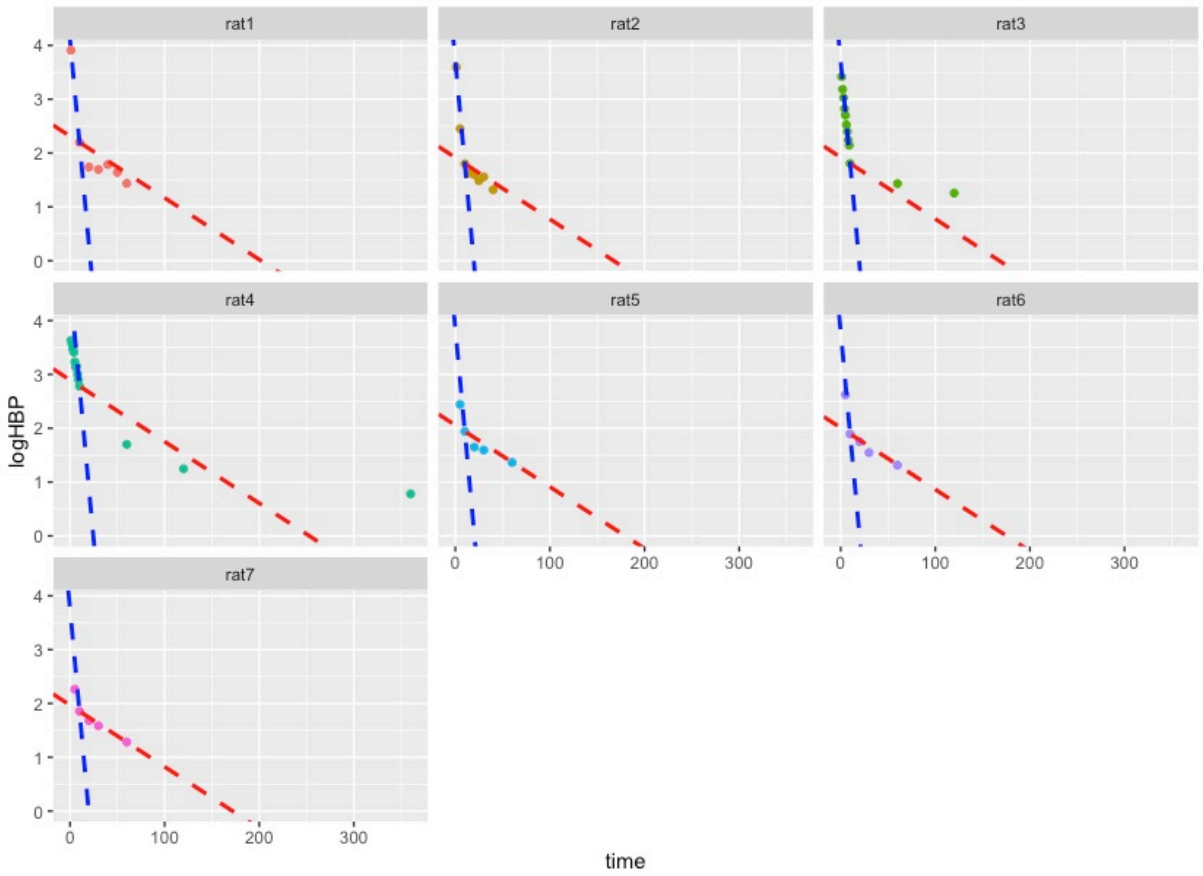
Sample	Background signal (1:40 dilution)	Background signal (1:400 dilution
plasma	0	
liver	21.8 ± 15.8	0.9 ± 1.6
spleen	0	
kidney	0	
lung	0	
urine	0	

Recovery was considered acceptable if the mean recovery was between 80-120%. According to these results we analyzed all rat plasma and lung, kidney, and spleen lysates and urine at a minimum dilution of 1:40. Because liver lysate produced a background signal in the ELISA at a 1:40 dilution which was mostly removed at 1:400 dilution, we ran all liver lysates at a dilution of at least 1:400.

Pharmacokinetic analyses



Supplementary figure S1. **Plasma HBP levels over time in rats injected intravenously with recombinant human HBP at a dose of 160 µg/kg.** Pharmacokinetic graphs for each individual rat with time in minutes vs log HBP. HBP values were log-transformed and the alpha phase (≤10 min) and beta phase (≥10 min) were modelled using linear regression. The blue line represents the estimated mean alpha phase and the red line the mean beta phase. The lines were modelled with the value at 10 minutes for each individual rat as a fixed value. The alpha phase was also modelled not subtracting the predicted beta phase from the values and the green line represents the estimated mean alpha phase from this calculation.



Supplementary figure S2. **Plasma HBP levels over time in rats injected intravenously with recombinant human HBP at a dose of 320 µg/kg**. Pharmacokinetic graphs for each individual rat with time in minutes vs log HBP. HBP values were log-transformed and the alpha phase (≤10 min) and beta phase (≥10 min) were modelled using linear regression. The blue line represents the estimated mean alpha phase and the red line the mean beta phase. The lines were modelled with the value at 10 minutes for each individual rat as a fixed value.

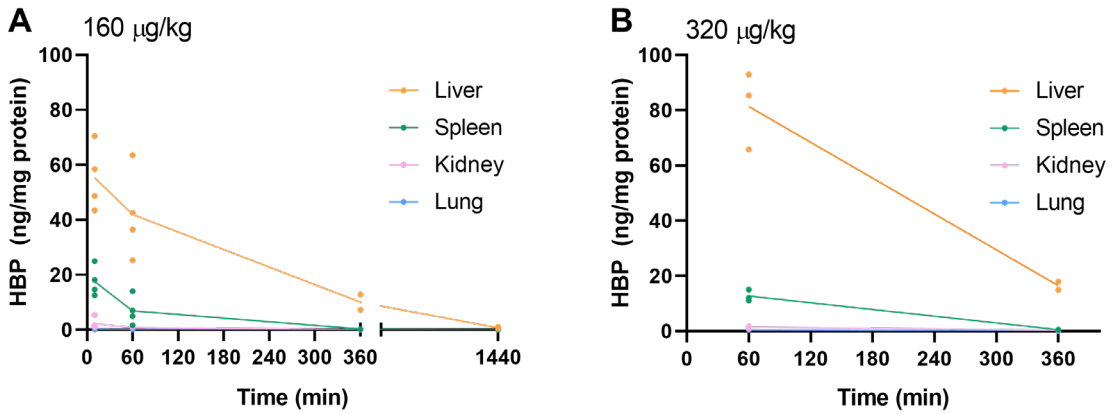
Supplementary table S3. Pharmacokinetic parameters for rats injected with 160 µg/kg HBP were calculated for each individual rat. HBP values were log-transformed and the beta phase (≥10 min) was modelled using linear regression. The fitted values were then subtracted from the alpha phase (0-10 min) and the alpha phase was fitted using linear regression. The alpha phase was also modelled not subtracting the predicted beta phase from the values.

Rat	k beta	k alpha	K alpha without subtraction of beta phase
1	NA	-	-0.178
2	NA	-	-0.205
3	NA	-	-0.223
4	NA	-	-0.185
5	-0.00899	-0.0816	-0.0906
6	-0.00629	-0.156	-0.163
7	NA	-	-0.108
8	NA	-	-0.117
9	-0.00889	-0.106	-0.115
10	-0.00529	-0.0959	-0.101
11	-0.00888	-0.0886	-0.0975
12	-0.00877	-0.101	-0.110
Mean	-0.00899	-0.0816	-0.178

Supplementary table S4. Pharmacokinetic parameters for rats injected with 320 µg/kg HBP were calculated for each individual rat. HBP values were log-transformed and the beta phase (≥10 min) was modelled using linear regression. The fitted values were then subtracted from the alpha phase (0-10 min) and the alpha phase was fitted using linear regression.

Rat	k beta	k alpha
1	-0.0115	-0.179
2	-0.0139	-0.182
3	-0.00493	-0.160
4	-0.00464	-0.0892
5	-0.0103	-0.0897
6	-0.0114	-0.134
7	-0.011	-0.0707
Mean	-0.0115	-0.179

HBP levels in organs over time



Supplementary figure S3. Rats were injected with recombinant human HBP at a dose of A)160 µg/kg and b) 320 µg/kg. HBP levels normalized for total protein concentration in lysates of liver, spleen, kidney, and lung are shown at 10 minutes (n=4 for 160 µg/kg), 60 minutes (n=4 for both), 6 hours (n=2 for both) and 24 hours (n=2 for 160 µg/kg). Data points are measurements from each individual rat, the colored lines connect the means at each time point.