Supplementary

Isolation of porcine hepatocytes

Hepatocytes were isolated from male Bama miniature pigs using a modified three-step collagenase perfusion method as described elsewhere. In brief, the liver was infused with 5 L of perfusate I (NaCl 8.3 g/L, KCl 0.5 g/L, HEPES 2.4 g/L, EGTA 0.95 g/L, NAC 0.8 g/L (Sigma-Aldrich, CA, USA)) at a flow rate of 400 ml/min Then the liver was excised and infused with 1.2 L of perfusate II (NaCl 8.3 g/L, KCl 0.5 g/L, HEPES 2.4 g/L) at the same speed further. Finally, 2 L of perfusateIII (NaCl 3.9 g/L, KCl 0.5 g/L, HEPES 24 g/L, CaCl₂ 2H₂O 0.7 g/L, BSA 2 g/L, collagenase NB 8 standard grade 120 PZ U/L (Serva, KG, Germany)) at 37 °C at a flow rate of 500 ml/min for 35 min recycle perfusion. Hepatocytes were washed with ice-cold wash media (Williams' Medium E 10.8 g/L, NaHCO₃ 2.2 g/L, HEPES 2.6 g/L, streptomycin 0.1 g/L, penicillin 100000 U/L, and 10% fetal bovine serum (FBS, Invitrogen, Carlsbad, CA)).

Hepatocyte-HUVEC organoids formation

To create the optimal hepatic organoids, fresh isolated porcine hepatocytes $(5 \times 10^{6}/\text{mL})$ and HUVECs were mixed in ratios of 1:10; 1:100 and 1:1000 in 20 ml of serum-free medium (SFM), and inoculated into spheroid dishes $(13 \times 8 \times 4 \text{ cm})$ made with glass and surface-siliconized with Sigmacote (Sigma, UK) respectively. Sealing strips on dishes were cut into several pieces to allow for gas exchange. Spheroid dishes were incubated at 37° C in a humidified atmosphere, supplied with 5% carbon dioxide gas mixture, and rocked continuously at 0.12 Hz for 24 h. The ratio of 1:100 got the best morphology of hepatic organoids with longer viability and functionality.

Induction of ALF in rhesus monkeys

ALF was induced in the rhesus monkeys with intraperitoneal administration of α -amanitin (Sigma-Aldrich, CA, USA) and lipopolysaccharide (LPS, Sigma-Aldrich, CA, USA) as described elsewhere. Before their administration, blood biochemical parameters of the liver and kidney were measured as baseline values. Then α -amanitin and LPS were mixed in 50 mL normal saline and slowly administrated intraperitoneally at a dose of 0.1 mg/kg body weight and 1.0 µg/kg body weight, respectively. Animals were then allowed to move and eat freely in cages.

SRBAL treatment of ALF monkeys

Before connected to the animals, the tubing set of blood circuit was primed with heparinized saline, while the ultrafiltration side was primed with treatment medium modified from SFM. As outlined in **Figure 1A**, the blood circuit was pumped at a rate of 20 mL/min for duration of 6 h. With the treatment prolonged, the animals regained the coagulation ability. There were increased risks of blood clotting in the pipeline. Heparin was regularly administered at a dose of 40 U/kg/h into the blood circuit and a bolus was given to keep the ACT ranging from 200-300 s to avoid the coagulation, then discontinued 30 min before the end of the treatment. Polygeline infusion was administered at the rate of 5-10 ml/min as a fluid expander if necessary to maintain the mean artery pressure (MAP) above 55 mmHg. Animals also received 250 mL normal saline to ensure hydration. D5NS and 50% dextrose were administered if necessary to raise the blood sugar. Excess fluid was removed via the SRBAL if fluid overload was observed during the SRBAL treatment.

Pig α -antitrypsin evaluation

Pig α 1-antitrypsin (α 1-AT) levels were measured using an ELISA kit (Bangyi, Shanghai, China), then analyzed with a MQX 200 microplate reader (BioTek Instruments Inc., VT, US). The α 1-AT levels in monkey blood gradually elevated during the SRBAL treatment. However, the α 1-AT levels dropped after treatment, and went back just above the baseline at 168 h after toxin infusion.

Sensitivity of PCR

The PCR products of PERV DNA fragment were cloned into TA vector and sequenced. Recombinant plasmids with PERV DNA fragment were constructed. Different copies of plasmids were amplified using Taq PCR Master Mix Kit (Qiagen, CA, USA) in accordance with the manual. The products were loaded on 2% agarose gel and visualized by the GoldView nucleic acid staining solution. Copies under 5×10^1 DNA were undetectable by PCR.



Figure S1. Dynamic changes in the vital signs of ALF monkeys. Vital signs including heart rate (HR), respiratory rate (RR), mean artery pressure (MAP), blood pH, partial pressure of oxygen (pO₂) and glucose (GLU).

		Unit	1st hour	2nd hour	3rd hour	4th hour	5th hour	6th hour
	RBC	10 ¹² cells/L	3.0±0.1	3.7±0.7	3.7±0.6	3.9±0.7	3.8±0.7	3.6±0.5
	HGB	g/L	90.3±2.3	98.1±9.3	99.5±8.6	103.5±9.4	100.7±7.9	96.0±5.3
	PLT	10 ⁹ cells/L	169.2±15.4	259.3±84.8	282.5±109.4	295.0±115.6	322.6±145.8	319.5±146.2
	ALT	IU/L	36.3±4.6	68.2±9.4	190.0±21.8	197.3±22.3	427.4±35.1	868.3±85.1
	AST	IU/L	28.5±3.8	176.3±23.4	385.0±55.3	400.3±58.2	548.5±52.3	1315.0±62.9
Sham group	ALB	g/L	30.7±0.9	30.9±0.8	30.8±0.9	31.9±0.9	31.4±0.9	30.8±0.9
	ТВ	μΜ	2.0±0.2	2.1±0.2	5.2±2.9	5.4±3.1	5.3±3.0	5.2±2.9
	Ammonia	μΜ	171.7±6.6	173.3±9.7	177.2±6.3	180.2±7.8	191.1±6.0	199.6±2.8
	BUN	mM	4.0±0.3	4.2±0.3	4.4±0.5	4.6±0.5	4.5±0.5	4.4±0.5
	S-100 β	ng/mL	2.1±0.1	2.0±0.1	2.1±0.1	2.2±0.1	2.1±0.1	2.1±0.1
	IgG	mg/mL	4.8±0.1	4.8±0.1	4.7 <u>±0.2</u>	4.9±0.2	4.8±0.2	4.7±0.2
	IgM	mg/mL	9.5±0.4	9.0±0.4	9.0±0.4	9.3±0.5	9.2±0.4	9.0±0.4
	RBC	10 ¹² cells/L	3.2±0.2	3.3±0.3	3.3±0.2	3.5±0.2	3.4±0.2	3.5±0.3
	HGB	g/L	93.8±1.8	95.6±3.4	95.2±2.7	98.7±2.2	97.1±2.7	96.8±3.7
	PLT	10 ⁹ cells/L	175.7±19.3	177.0±18.6	176.0±19.6	183.4±21.5	179.6±20.0	150.8±19.9
	ALT	IU/L	14.5±4.3	37.9±11.6	137.3±15.0	142.3±15.0	158.3±3.4	303.3±36.1
	AST	IU/L	24.5±4.6	134.7±21.8	103.8±13.1	107.7±13.5	169.9±6.8	361.7±85.2
group A	ALB	g/L	32.2±2.5	32.3±2.5	32.3±2.5	33.6±2.7	33±2.6	30.1±1.7
	ТВ	μΜ	2.0±0.1	2.0±0.1	2.5±0.3	2.6±0.3	2.5±0.3	3.5±0.6
	Ammonia	μΜ	86.1±6.3	99.5±5.3	105.8±4.9	109.0±4.7	106.9±5.9	111.2±4.5
	BUN	mM	3.7±0.3	3.8±0.3	4.5±0.5	4.7±0.5	4.6±0.5	4.8±0.3

Table S1. Laboratory values during the SRBAL treatment in sham group and groups A, B, C.

	S-100 β	ng/mL	2.0±0.0	2.0±0.0	2.1±0.0	2.1±0.0	2.1±0.0	2.1±0.0	
	IgG	mg/mL	5.1±0.2	5.2±0.1	5.2±0.2	5.4±0.2	5.3±0.2	5.2±0.2	
	IgM	mg/mL	14.9±2.5	16.0±1.0	19.2±1.9	21.3±2.2	22.8±1.2	25.9±1.2	
	RBC	10 ¹² cells/L	2.8±0.1	2.9±0.2	2.9±0.2	3.0±0.2	3.0±0.2	2.9±0.2	
	HGB	g/L	84.2±1.2	85.7±1.6	87.2±1.4	90.5±1.7	88.9±1.4	87.2±1.4	
	PLT	10 ⁹ cells/L	177.2±18.1	177.3±17.9	176.2±18	183.4±19.9	179.8±18.4	176.2±18.0	
	ALT	IU/L	42.2±6.1	95.1±6.0	168.8±13.9	175.7±15.5	338.9±23.1	606.7±43.9	
	AST	IU/L	243.0±14.6	1096.1±132.3	1125.0±100.3	1166.9±102.0	1296.1±65.7	1476.7±86.5	
group B	ALB	g/L	22.6±1.2	22.8±1.2	24.4±1.6	25.3±1.7	24.9±1.7	24.4±1.7	
	ТВ	μΜ	2.1±0.2	2.1±0.2	2.5±0.4	2.6±0.4	2.6±0.4	3.8±1.6	
	Ammonia	μΜ	184.7±12.4	188.0±10.1	201.9±9.2	204.8±6.9	210.6±7.3	216.9±8.5	
	BUN	mM	4.1±0.2	4.1±0.2	4.0±0.2	4.1±0.2	4.0±0.2	4.0±0.2	
	S-100 β	ng/mL	4.0±0.1	4.4±0.3	4.8±0.3	5.1±0.4	6.5±0.3	6.9±0.2	
	IgG	mg/mL	5.6±0.2	5.3±0.3	5.5±0.2	5.6±0.2	5.4±0.3	5.5±0.2	
	IgM	mg/mL	27.4±5.4	28.6±5.5	29.1±4.2	31.0±2.8	32.8±1.1	33.2±1.9	
	RBC	10 ¹² cells/L	2.9±0.1	2.9±0.1	2.9±0.1	3.0±0.2	2.9±0.2	2.8±0.2	
	HGB	g/L	70.3±3.2	71.4±3.5	73.8±3.8	76.6±3.8	71.9±4.3	71.3±4.2	
	PLT	10 ⁹ cells/L	178.0±11.7	176.0±10.5	180.7 ± 10.5	187.3±10.2	169.6±16.8	164.0±22.5	
	ALT	IU/L	47.3±5.9	111.8 ± 10.5	279.7±29.3	289.8±29.8	474.5±123.9	726.7±30.3	
	AST	IU/L	118.1 ± 18.2	571.9±25.5	1017.1±66.9	1054.1±64.8	1412.8±85.8	1708.3±58.0	
group C	ALB	g/L	25.5±3.3	22.9±0.6	28.8±3.7	30.1±4.1	27.2±4.3	30.5±5.2	
	TB	μΜ	2.0±0.3	1.9±0.2	2.4±0.2	2.5±0.2	2.8±0.6	3.1±0.7	
	Ammonia	μΜ	235.1±8.3	295.8±6.6	316.3±8.9	326.0±7.5	335.8±4.9	348.9±5.9	
	BUN	mM	7.4±0.6	6.8±0.7	7.4±0.6	7.6±0.6	6.9±0.8	6.0±0.8	
	S-100 β	ng/mL	4.8±0.2	5.2±0.6	6.4±0.6	7.2±0.4	8.5±0.2	9.1±0.2	
	IgG	mg/mL	5.2±0.2	5.2±0.3	5.7±0.2	5.7±0.1	5.4±0.2	5.5±0.2	
	IgM	mg/mL	35.7±1.8	37.8±0.9	38.9±1.4	37.6±1.6	39.0±3.1	39.5±3.4	



Figure S2. Viability Fluoroquench staining of hepatocyte organoids after 6-hour SRBAL treatment (green- alive cells; red- dead cells).



Figure S3. Oxygen consumption of hepatocyte organoids before and after 6-hour SRBAL treatment. *p < 0.05.



Figure S4. Ammonia clearance of hepatocyte organoids before and after 6-hour SRBAL treatment. *p < 0.05.



Figure S5. The albumin permeability test of 65 kD hollow fiber cartridge membrane. The tubing set of blood circuit was primed with treatment medium with 30 g/L albumin, while the ultrafiltration side was primed treatment medium with 5 g/L albumin.



Figure S6. The α -amanitin concentration and kinetics in systemic plasma in toxin-induced monkey model.



Figure S7. The α-amanitin concentration in systemic plasma before and after SRBAL treatment.



Figure S8. The α-amanitin concentration in SRBAL waste line before and after SRBAL treatment.



Figure S9. The pig albumin concentration in SRBAL treatment medium and monkey blood.



Figure S10. The pig α 1-antitrypsin (α 1-AT) concentration in SRBAL treatment medium and monkey blood.

Table S2 Primer sequences for droplet digital PCR

Gene	Forward Primers	Reverse Primers				
SLA-DR	CCCTGACAAATCTGGCGAGT	GGCTTGTCTGAGAGCACAGT				
SLA-2	AGCCATCCTCATTCTGCTGT	ATCCCAATAGTCCTGCCCCT				
Cyt B	ACGCATTCATTGACCTCCCA	TGCTCCGTTTGCATGTAGGT				



Figure S11. Droplet digital PCR results of SLA-DR from porcine hepatocytes and monkey PBMCs and liver.



Figure S12. Droplet digital PCR results of SLA-2 from porcine hepatocytes and monkey PBMCs and liver.



Figure S13. Droplet digital PCR results of Cyt B from porcine hepatocytes and monkey PBMCs and liver.

hepatocyte negative positive	marker	PK15 positive	5×1010	5×10 ⁸	5×10 ⁶	5×104	5×10 ³	1×10 ³	5×10²	1×10 ²	5×101	5×10º
1 2		4							11	12	13	14
	2000bp											
	1000bp											
	750bp											
	500bp											
	250bn											
	20000		-	-	-							
	100bp											

Figure S14. Sensitivity of PCR. Sample 1, 2, 4: negative and positive control from ddH_2O , porcine hepatocyte, PK15 cell line; Sample 5-14: different copies of recombinant plasmids with PERV DNA fragment. Copies under 5×10^1 DNA were undetectable by PCR.

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Approval of Animal Ethical and Welfare
* 包骥 教授
经四川大学华西医院实验动物伦理委员会初审和复审审查后,您申报的课题
使用生物人工肝设备治疗急性药的肝肠伤恒河推横型的研究
符合国家实验动物语利伦理的相关相定 绘予通过
Dear Professor Li Baro
Animal Ethics Committee have a meeting as scheduled, the participators have
evaluated the care and use of animals described in the protocol
of evaluation of bipartitical liver with percine hepitoples in chesus monkey of ALF
and find the procedures described as appropriate and acceptable.
Approval No 2016063A
ALL
华西医院实验动物伦理委员会主席
Signature of Chairman
时间 Date 2015、01.06

Ethical statement