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Xenogeneic ex vivo hemoperfusion of rhesus monkey livers with human blood

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Abstract In order to copy the clinical situation of concordant xenotransplantation, Rhesus Monkey livers were hemoperfused with human blood. Changes of immunological (TNFα, IL-1β, IL-2, IL-2R, IL-6, IFNγ, TXB2, 6kPGF1α, sICAM-1, sELAM-1, sHLA-I-Ag) and pathophysiological (GOT, GPT, LDH, CK) parameters were followed. Our experiment proves that all phenomena start in the first hour of xenogeneic blood circulation. Xenogeneic rejection in our concordant system is surprisingly severe. Preformed natural antibodies only cannot be the reason of such a damage [5, 6]. We think that beside other important immunological mechanisms, humoral mediators play a considerable role at the beginning of a xenogeneic rejection.

Key words Xenograft · Ex vivo hemoperfusion · Mediators Cytokines · Eicosanoids Adhesion molecules · sHLA I Ag Perfusion circuit · Xenogeneic rejection · Primate · Liver

Introduction

The lack of human donor organs and the rapid increase of patients on the waiting lists force scientists to look for new recources of grafts. Xenotransplantation would be the most promising possibility [1, 2, 3]. The past shows, however, that there are major problems to protect recipient and donor organs from xenogeneic rejection [4]. Such clinical experiments using closely related donors and human recipients pose the question which reactions reduce the viability of a xenograft.

Methods

After intramuscular premedication and deep anesthesia male Rhesus Monkeys (RM) ($n = 6$; 10.3 ± 0.6 kg) were hepatectomized. Using common surgical techiques to mobilize the livers (178 ± 4 kg), they were flushed in situ with 4 °C UW solution via A. hepatica com. and V. portae and immediately explanted. During a cold ischemia of 90 ± 10 min while the livers had been prepared for fixing in the circuit, reperfusion started with pH stabilized, oxygenated and heparinized 37 °C warm fresh human blood of bloodgroup BG 0 and B. Blood has been diluted with 0.9 % NaCl solution, human albumin and dextrane 60 to 1.2 l of perfusate ($HB = 11.6 \pm 1.9$ g/dl; $Hct = 34.3 \pm 5.8$). BG A can crossreact with TM tissue [7]. The perfusion system [8] allows electronic control of blood flow, pressure and temperature. The liver was pressurized to mimic the changing intraabdominal diaphragmatic breathing pressure.

Blood was dialysed against a commercial solution to stabilize electrolytes. Over a period of 4 h venous effluates (EF) at (intervals of: 0, EF, 5, 10, 15, 30, 60, 120, 180 and 240 min) and bile production at intervals: 60, 120, 180 and 240 min) were measured. We declared the status before reperfusion as 0 min. The circuit was closed after 300–350 ml of blood had flushed the livers. This volume was discarded for further investigation, i.e. aliquots used as effluate (EF). Plasma specimens were stored at −80 °C.

Liver-specific enzymes [9], including urea and uric acid, O_2 consumption, blood gas, pH and electrolytes were determined [10]. Immunological effects of cytokines [TNFα, IL-6, IL-2, IL-2R, IL-1β, IFNγ (pg/ml)] [11–13], soluble adhesion molecules (sAM) [ICAM-1, ELAM-1 (ng/ml) [14–16] and soluble HLA-I-antigens (pg/ml) [17] were secured by ELISA and eicosanoids [TXB2,

6kPGF1α (pg/ml)], by RIA. Measurement of the enzymes, GOT, GPT, LDH and CK (U/ml) was by common biochemical clinical tests. Samples of similar blood perfusing the circuit without an organ ($n = 5$) were collected at the same intervals as controls.

Results

The parameters monitored are shown in Table 1; and results are given for the relevant time points (mean ± SEM). The massive changes up to 15 and 60 min indicated early severe interactions of human blood with RM liver endothelium and hepatocytes. The increase in TNFα, IL-1β, IL-6 and IFNγ up to 30 min after reperfusion was of particular interest. Later, a dramatic increase in these parameters, which are functionally coherent was seen. IL-2 increased after 60 min. Prior to that no IL-2 was detected. In the controls, no IL-2 was detected. The soluble IL-2 receptor decreased to about 50% of initial value up to 60 min. After 180 min it increased rapidly by more than 100%. During perfusion, there were no significant changes in TXB2. 6kPGF1α started to increase dramatically after 60 min. The controls showed no major change. (sAM) sELAM-1 and sICAM-1 and soluble HLA-I antigen showed a decrease to less than 50% of their initial values. GOT, GPT and LDH were released in the first 15 min after reperfusion. After that, no significant increase in GOT and GPT were detected. LDH increased slowly after 15 min until the end of perfusion. Surprisingly, creatine kinase (CK) increased constantly
during isolated haemoperfusion to a pathological level at 180 min after reperfusion. Controls showed no major change (Table 2).

Table 1 Values of humoral mediators and enzymes released from rhesus monkey livers during xenogeneic perfusion with human blood. Mean values of healthy volunteers (MN) are given by the companies. (Quantikine, BBE, Cellfree by Hermann Biermann GmbH Diagnostica) For biochemical enzyme tests normal mean values refer to the Institute for Clinical Chemistry LMU Großhadern

Time	TNFα	IL-1β	IL-2	IL-2R	IL-6	IFNγ	PGF1α	TXB2
0 min	367±271	13±9	0±0	411±130	5±1	79±49	177±33	1469±146
EF	850±203	17±10#	0±0	329±91	13±4#	53±33	648±124#	1746±407
5 min	679±134*	16±9#	0±0	355±108	18±3*#	137±78	585±85*#	1641±295*
10 min	704±160	12±5	0±0	282±82	19±2#	111±61	463±51#	1581±277
15 min	699±164*	15±5	0±0	236±72#	34±7*#	107±53	454±49*#	1448±227*#
30 min	987±326*	17±5	0±0	290±73#	81±15*#	89±48	336±12*#	1612±327*#
60 min	1907±460*#	63±18*#	0±0	257±50*	500±44*#	144±99	2837±1055*#	1530±322*
120 min	2412±467#	379±87*#	91±31*#	234±69*#	564±48*#	425±186*#	5364±170*#	1356±207#
180 min	2586±612#	483±76*#	223±71*#	243±53*#	619±45*#	555±176*#	6483±231*#	1658±237*#
240 min	1868±565#	586±81*#	371±93*#	744±210*	681±59*#	1218±358*#	6826±231*#	1776±256#
MN	<25.8	<3.8	<31.3	<573	<6.25	<20		

Table 1 (continued)

Time	sICAM1	sELAM1	sHLAI	GOT	GPT	LDH	CK
0 min	162±32	47±7	798±221	8±1	11±3	129±19	27±4
EF	142±17	41±6	615±176#				
5 min	126±12	37±6#	629±188				
10 min	122±20#	41±8	620±173				
15 min	116±15#	44±7	541±140	40±10#	37±8#	251±22*#	37±5#
30 min	112±13	36±6#	536±168#	43±9*#	38±7*#	271±13*#	42±5#
60 min	116±22	34±6#	517±147#	42±10*#	37±8*#	280±24*#	51±7*#
120 min	108±17#	31±6#	468±133#	48±18*#	36±11*#	285±27#	53±8*#
180 min	90±10#	28±5#	444±114#	55±26*#	37±15*#	319±47#	74±31*#
240 min	77±15#	23±6#	358±90#	59±28*#	38±16*#	335±50#	83±41#
MN	<286	<66.7	<1.6	5–17	5–24	80–240	<80

* $P < 0.05$ (U test): Perfusion of Rhesus Monkey livers in the circuit as compared to blood flow in the circuit without an organ during the same time period (Wilcoxon rank sum)

\# $P < 0.05$ (Wilcoxon test): Shows the significant differences between 0 min and the point of messuring

Table 2 Values of the release of humoral mediators and enzymes in the controls (The reaction between the used materials in the circuit and similar prepared human blood)

Time	TNFα	IL-1β	IL-2	IL-2R	IL-6	IFNγ	PGF1α	TXB2
0 min	14±12	3±1	0±0	363±69	4±1	32±16	30±29	182±141
15 min	10±8	5±2	0±0	487±107	3±1	23±11	36±25	305±173
30 min	12±9	5±2	0±0	444±69	4±1	22±9	7±3	407±212
60 min	201±87	9±3	0±0	495±114	7±4	28±13	7±2	762±182
120 min	958±324	21±7	0±0	689±152	73±41	23±9	16±8	985±208
180 min	1618±290	33±7	0±0	723±123	312±93	32±13	47±21	1130±227
240 min	1860±290	42±6	0±0	673±106	520±65	42±19	163±48	1216±248
MN	<25.8	<3.8	<31.3	<573	<6.25	<20		

Table 2 (continued)

Time	sICAM1	sELAM1	sHLAI	GOT	GPT	LDH	CK
0 min	75±17	15±5	6±1	5±1	3±1	122±15	25±7
15 min	63±9	13±4	5±1	5±1	3±1	127±10	26±8
30 min	68±16	13±5	6±1	5±1	3±1	141±14	26±8
60 min	64±12	16±6	6±1	5±1	3±1	160±20	26±8
120 min	72±13	16±5	6±1	6±1	3±1	191±35	25±7
180 min	80±19	16±6	6±1	6±1	3±1	230±46	25±7
240 min	86±18	14±5	6±0	7±1	4±1	264±55	26±7
MN	<286	<66.7	<1.6	5–17	5–24	80–240	<80

Discussion

This model of isolated ex vivo haemoperfused RM livers reflected immunological effects of donor organs and the humoral blood components of the recipient. The reaction between the isolated organ and the recirculating blood allowed us to concentrate on the effects and products liberated under such a selected but limited situation. The influence of the materials used in the circuit on the parameters monitored was of no importance.

The massive release of TNFα, IL-1β, IL-6, IFNγ and 6kPGF1α between 15 and 60 min after reperfusion was the specific result of xenoperfusion. IL-2 increased after 60 min. Soluble human IL-2R seemed to be eliminated from the perfusate by the RM liver to about 50% of initial values. It increased again sharply after 180 min at this stage probably originating from the RM liver. During the whole perfusion time, soluble HLA-I-Ag and the sAM decreased to 50% of their initial values.

The increase in GOT, GPT and LDH in the first 15 min seemed to be the result of ischaemic damage before reperfusion. After that point, these parameters reflected the small amount of cellular damage in the livers during perfusion. However, the constant increase in CK during perfusion could be more important sensitive physiological parameter for liver cell necrosis or endothelial damage [18] than the other parameters. We determined that the isoenzyme CK-MB increases CK in the perfusate. CK was chosen as an important liver parameter despite being a common muscular and myocardiological parameter [19].

From our experiments, it appeared that humoral mediators in our concordant system reflected a massive interaction between RM liver endothelium, hepatocytes and unmodified human blood. The severity of xenogeneic interactions in this closely related primate system exceeded by far that of allografts. Monitoring of the beginning of the humoral rejection with these assays was very sensitive. CK as a new liver-specific physiological parameter in isolated systems could be used as a sensitive measurement of hepatic cell necroses.

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