Title : ROLE OF THROMBOXANE A_2 DURING INCOMPATIBLE HOMOLOGUOUS AND HETEROLOGUOUS BLOOD TRANSFUSION IN SHEEP

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Introduction. Although rarely observed today, adverse reactions to blood transfusions may occasionally present a different clinical picture than the classical triad: hemolysis, disseminated intravascular coagulation, and acute renal failure. Non cardiogenic pulmonary edema associated with pulmonary hypertension and severe hypoxemia have been reported as well (1-3). Transfusional reactions activate the complement system, and complement-derived anaphylatoxins have been shown to stimulate the synthesis of prostaglandins and thromboxane A_2 (4), both metabolites resulting from the cyclooxygenation of arachidonic acid. Thromboxane A_2 is a potent vaso- and bronchoconstrictor in several species, including man, and therefore may possibly be held responsible for the pulmonary hypertension observed during transfusional reactions. The aim of the present study was to analyze systemic and pulmonary hemodynamics and the biologic response (circulating cells, thromboxane A_2 production) to the transfusion of incompatible blood in an experimental animal, and to evaluate the preventive effect of the cyclooxygenase inhibitor indomethacin on this reaction.

Materials and Methods. Adult sheep (weight 30-40 kg) were surgically instrumented for chronic studies with vascular catheters introduced into the thoracic aorta, pulmonary artery, and right and left atrium. Through a left thoracotomy, a transit-time ultrasonic blood flow probe was placed around the main pulmonary artery for continuous determination of cardiac output. Vascular pressures were continuously measured using calibrated pressure transducers positionned at mid-thoracic level and connected to a 6-channel recorder. Systemic and pulmonary vascular resistances were calculated by standard formulae. Circulating platelets and leukocytes were measured by phase microscopy. Free hemoglobin concentration in blood was measured by spectrophotometry. Plasma levels of TxB_2 were determined by standard radioimmunoassay. The studies were carried out at least three days after the surgical preparation, with the animals standing in a specially adapted cage for chronic studies. In preliminary studies in four sheep, we found that infusion of sterile incompatible autologous (sheep) blood produced an isolated and moderate pulmonary hypertension. In order to enhance the evoked adverse response to incompatible blood in this species, we then designed to evaluate the effects of heterologuous blood infusion (human blood provided from the hospital's blood bank, stored in citrate-phosphate-dextrose at 4°C, at the most during 3 weeks) in the same sheep, as well as in five additional sheep. On separate experimental days, the sheep received a 60 ml transfusion of homologuous or heterologuous blood warmed up to 37°C and injected into the right atrium at a constant rate (20 ml/min). The incompatible blood (homologuous or heterologuous) of a same donor was used for one animal. The following experimental conditions were randomly investigated: A) transfusion of homologuous blood, n=4; B) transfusion of heterologuous blood, n=9; C) transfusion of heterologuous blood preceeded by pre-treatment with 2mg/kg indomethacin administered i.v. during the 30 min immediately before the start of transfusion, n=7. Statistical comparison over time and between treatment groups was conducted by a one-way ANOVA for repeated measurements, with significant ($P<0.05$) differences detected by Duncan's multiple comparison test. Where appropriate, data were analyzed after transformation to a logarithmic scale, to satisfy constant variability.

Table 1. Hemodynamic Data

Variable	Group	Time (minutes)						P
		-3	1	3	5	10	30	
MAP	A	84 ± 3	91 ± 5	98 ± 7	101 ± 8	87 ± 3	85 ± 3	NS
(mm Hg)	B	79 ± 4	71 ± 8*	104 ± 26	160 ± 17*	107 ± 7*	97 ± 3	0.06
	C	84 ± 2	83 ± 4	91 ± 8	117 ± 14	104 ± 4*	88 ± 3	0.005
MPAP	A	21 ± 2	28 ± 2	34 ± 2	32 ± 2	26 ± 1	21 ± 1	< 0.0001
(mm Hg)	B	20 ± 2	35 ± 3	34 ± 4	34 ± 4	28 ± 6	23 ± 10	0.005
	C	19 ± 2	28 ± 5	35 ± 5	31 ± 7	34 ± 3	32 ± 2	0.01
CO	A	4.6±0.3	4.4±0.4	4.3±0.3	4.5±0.3	4.8±0.4	5.2±0.5	NS
(L/min)	B	4.9±0.6	1.2±0.3*	1.3±0.4*	3.2±0.4	2.6±0.3*	4.0±1.1	0.0002
	C	4.3±0.7	3.6±0.6	3.5±1.0	4.6±0.8	4.4±0.6	4.4±0.6	NS
CVP	A	3 ± 1	4 ± 1	5 ± 1	4 ± 1	3 ± 1	3 ± 1	NS
(mm Hg)	B	5 ± 2	27 ± 4*	22 ± 6*	5 ± 2	5 ± 4	2 ± 0	< 0.0001
	C	7 ± 1	7 ± 2	11 ± 4*	6 ± 2	4 ± 2	7 ± 1	NS
LAP	A	5 ± 3	8 ± 3	8 ± 3	5 ± 3	6 ± 3	6 ± 2	NS
(mm Hg)	B	9 ± 2	12 ± 6	23 ± 5	19 ± 8	18 ± 2	13 ± 0	NS
	C	4 ± 2	4 ± 2	3 ± 3	4 ± 3	2 ± 3	3 ± 3	NS
SVR	A	18 ± 1	22 ± 2	23 ± 2	22 ± 2	18 ± 2	17 ± 2	NS
(units)	B	17 ± 3	104 ± 31*	98 ± 18*	53 ± 9*	40 ± 4*	25 ± 6	< 0.0001
	C	21 ± 3	25 ± 3	29 ± 13	34 ± 10	27 ± 6	20 ± 2	NS
PVR	A	4 ± 1	6 ± 1	8 ± 1	7 ± 1	5 ± 1	4 ± 1	NS
(units)	B	3 ± 1	54 ± 27*	16 ± 10	5 ± 3	5 ± 3	4 ± 0	0.007
	C	4 ± 1	8 ± 2	16 ± 1	9 ± 3	11 ± 4	8 ± 2	NS

MAP:mean arterial pressure; CVP:central venous pressure; LAP:left atrial pressure; MPAP:mean pulmonary arterial pressure;CO:cardiac output;SVR:systemic vascular resistance;PVR:pulmonary vascular resistance Group A:Homologuous blood, B:Heterologuous blood, C:Heterologuous blood + Indomethacin
$\bar{x}$ ± SE; *$P < 0.05$ significantly different from group A

Table 2. Plasma Thromboxane B2 (TxB2), Free Hemoglobine (Hb), and White Blood Cell (WBC) Count

Variable	Group	Time (minutes)						P
		-3	1	3	5	10	30	
TxB_2	A	0.7±0.2	3.4±2.0	3.9±2.6	1.2±0.3	1.2±0.5	·	0.07
(ng/ml)	B	0.6±0.2	129±52*	184±144*	81±46*	411±338*	·	0.0001
	C	0.3±0.1	0.5±0.4	1.8±1.2	0.6±0.1	1.4±0.4	·	NS
Free Hb	A	5.4±1.1	6.8±1.5	5.9±1.0	5.5±0.8	6.4±1.2	6.4±1.3	NS
(mg/100ml)	B	4.8±2.0	49 ± 19*	81 ± 18*	68 ± 20*	56 ± 0*	52 ± 0*	0.0002
	C	3.5±0.7	29 ± 8*	50 ± 16*	52 ± 14*	50 ± 14*	43 ± 12*	0.0001
WBC	A	100 ± 0	93 ± 2	93 ± 5	96 ± 5	103 ± 5	·	NS
(% baseline)	B	100 ± 0	34 ± 12*	13 ± 3*	18 ± 4*	10 ± 2*	35 ± 0	< 0.0001
	C	100 ± 0	46 ± 11*	15 ± 4*	17 ± 5*	27 ± 8*	54 ± 7	< 0.0001

$\bar{x}$ ± SE; *$P < 0.05$ significantly different from group A

Results. The main results of this study are indicated on Table 1 and Table 2. Infusing homologuous, but incompatible blood, in the four sheep of group A produced only a transient pulmonary hypertension associated with a moderate increase in plasma TxB_2 levels. No other change in any of the measured variables was observed in this group, in particular, no significant increase of free hemoglobin concentration was found. In contrast, infusing heterologuous blood dramatically decreased cardiac output due to a intense pulmonary and systemic vasoconstriction, leading to death in 6 out of the 9 sheep within the first 5 min following the start of blood infusion. This reaction was associated with markedly elevated plasma TxB_2 levels, leukopenia, and hemolysis. Pretreating animals with indomethacin remarkably prevented death, the rise of TxB_2, and the acute hemodynamic, but not the biological response, since similar leukopenia and free hemoglobin concentration were measured in this group.

Discussion. Our results clearly demonstrate that cyclooxygenase metabolites, most probably the vasoconstrictor thromboxane A_2, are responsible for the acute cardiovascular disaster observed during uncompatible heterologuous blood transfusion.

References.

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