Blood Purification

Blood Purif 2019;47(suppl 3):51–53
DOI: 10.1159/000499546

Published online: April 12, 2019

Analysis of Endotoxin Adsorption in Two Swedish Patients with Septic Shock

Marcus Ewert Broman Mikael Bodelsson

Perioperative and Intensive Care, Skåne University Hospital, Lund, Sweden

Keywords
Sepsis · Acute kidney injury · Endotoxin

Abstract
Background: Lipopolysaccharide (endotoxin) from the outer Gram-negative bacterial wall can induce a harmful immunologic response, involving hemodynamic deprivation, and is one important motor driving the septic cascade. The positively charged poly-imine ethylene layer on the oXiris membrane is capable of adsorbing negatively charged endotoxin molecules and removing them from the blood compartment. Endotoxin is detrimental and should be removed from blood. ***Summary:*** The adsorbable endotoxin fraction in blood arises from a tight balance between seeding from an infectious focus and removal by an overwhelmed immune system. The net sum of remaining endotoxin in blood is available for an adsorption process in the oXiris filter. Endotoxin data from 2 patients with severe Gram-negative septic shock and endotoxemia in this case series, speaks for a considerable share of the adsorption of the oXiris filter in the endotoxin net removal over time. ***Key Messages:*** Analysis of combined in vitro and in vivo data speaks for an effect of the oXiris filter in lowering endotoxin.

The AN69 bulk based and amplified with a positively charged poly-imine ethylene layer capable of adsorbing negatively charged endotoxin molecules, is in use, for patients with critical septic shock and adjacent acute kidney injury requiring continuous renal replacement therapy at the general intensive care unit at Skåne University Hospital, Lund, Sweden. The oXiris filter is a full blown dialysis filter capable of providing a complete dialysis treatment in addition to its adsorbing ability [1]. Lipopolysaccharide (endotoxin) from the outer Gram-negative bacterial wall can induce a harmful immunologic response, involving hemodynamic deprivation in Gram-negative septic shock, and is one important motor driving the septic cascade. Endotoxin levels have been correlated to the severity of illness, maximum organ dysfunction, and mortality. Endotoxin can also occur in Gram-positive septic conditions, for example, by originating from an ischemic gut [2].

Endotoxin molecules have a size of about 10 kDa but can form aggregates up to 1,000 kDa, consisting of a lipid and polysaccharide covalently bonded. They attach to the toll-like receptor 4, especially in dendritic cells, monocytes, macrophages, and lymphocytes, which promotes the secretion of pro-inflammatory cytokines. Endotoxins are also strong pyrogens. They are to be considered as completely detrimental and induce an escalating shock

KARGER

E-Mail karger@karger.com
www.karger.com/bpu

Marcus Ewert Broman
Perioperative and Intensive Care
Skåne University Hospital
Getingevägen 4, SE–22241 Lund (Sweden)
E-Mail marcus.broman@med.lu.se

Table 1. Clinical data of the included patients A and B

Study patient study centre	Patient A	Patient B
Gender	Male	Male
Age, years	73	82
Source of infection	Cholecystitis	Colon perforation
Bacteria growth in blood	*E. coli*	None
SAPS3	77	67
SOFA	16	13
Filter	oXiris 24 h	oXiris 24 h
KDIGO class	3	3
Blood flow, mL/min	300	90
CRP, mmol/L	279	391
Prokalcitonin, μg/L	42	185
Krea, mmol/L	315	174
Urea, mmol/L	13.1	14.7

state in humans, which manifests as vasodilation, capillary leakage, relative hypovolemia, and hypotension.

The endotoxin load in the blood stream can exist in form of a short initial pulse, which is fast cleared by the intact immune system, but it can also exist in a tight balance between the clearance capacity of an overwhelmed failing immune system and a constant feeding by the infectious bacterium colony. Some foci might be more encapsulated and the endotoxin might not reach the blood easy, while others are more profoundly perfused and thus more prone to seed endotoxin [3]. Normally the immune system neutralizes the endotoxin within minutes; mainly in the liver. If the system is overwhelmed a harmful fraction remains in blood, which is available for an adsorption process when blood is pumped through the oXiris filter. Theoretically, the blood flow, the concentration of endotoxin, and the total inner surface of the filter are the most important factors determining the endotoxin reduction rate. After a while, the membrane will be saturated and a filter change must take place [3, 4].

In a former simplistic in vitro model [5], 15.8 μg was injected into the oXiris filter and 6.9 μg (43.7%) was removed over 6 h. We tried to find out the corresponding endotoxin reduction in 2 patients with severe Gram-negative septic shock including verified endotoxemia. Both patients showed KDIGO class 3 acute kidney injury, and continuous renal replacement therapy was started using an oXiris filter. Arterial blood samples were drawn at 0, 1, 3, 8, 16, and 24 h for endotoxin measurement in our research lab. Our updated Limulus Amebocyte Lysate assay was used [6]. Patient data and treatment data are presented in Table 1, and endotoxin data including calculations are presented in Table 2. For patient A, an estimated amount of 70,000 pg was present initially, and 45,000 pg (64.3%) was removed by the eighth hour measurement (Table 2). Correspondingly for patient B, an estimated amount of 35,000 pg was present initially and 20,000 pg (57.1%) was removed

Table 2. Endotoxin concentration reduction over 24 h and number of blood volume returns for patients A and B*

Time	Patient A	Patient B
0 h (baseline)	0.14 EU/mL 14 pg/mL 70,000 pg 0 turns	0.07 EU/mL 7 pg/mL 35,000 pg 0 turns
1 h	0.13 EU/mL[1] 13 pg/mL[2] 65,000 pg[3] –5,000 pg[4] –7.1%[5] 3.6 turns[6]	0.04 EU/mL 4 pg/mL 20,000 pg –15,000 pg –42.9% 1.1 turns
3 h	0.11 EU/mL 11 pg/mL 55,000 pg –15,000 pg –21.4% 10.8 turns	0.03 EU/mL 3 pg/mL 15,000 pg –20,000 pg –57.1% 3.2 turns
8 h	0.05 EU/mL 5 pg/mL 25,000 pg –45,000 pg –64.3% 28.8 turns	0.03 EU/mL 3 pg/mL 15,000 pg –20,000 pg –57.1% 8.6 turns
16 h	0 EU/mL 0 pg/mL 0 pg –70,000 pg –100% 57.6 turns	0 EU/mL 0 pg/mL 0 pg –35,000 pg –100% 17.3 turns
24 h	0 EU/mL 0 pg/mL 0 pg –70,000 pg –100% 86.4 turns	0 EU/mL 0 pg/mL 0 pg –35,000 pg –100% 25.9 turns

* 100 pg endotoxin gives 1 EU.
[1] Endotoxin concentration in blood.
[2] Endotoxin concentration in blood.
[3] Total endotoxin amount in blood.
[4] Total endotoxin removal since hour zero.
[5] Total endotoxin removal since hour zero.
[6] Blood volumes through filter.
EU, endotoxin unit.

by the eighth hour (Table 2). For the calculations of the absolute endotoxin amount from the measured endotoxin concentration, the assumption of a blood volume of 5 L was made. In the in vivo situation, many actors come into play; a variable seeding rate from the bacterium colony into the blood compartment, degradation by the overwhelmed immune system, and adsorption on the oXiris membrane. How these actors divide between themselves of the total net removal is impossible to calculate.

A theoretical formula describing the in vivo endotoxin balance could be:

endotoxin removal =
(endotoxin seeding from infectious focus)
– (endotoxin degradation by the immune system)
– (spontaneous endotoxin degradation)
– (adsorption on the oXiris membrane due to blood flow and due to modified Langmuir system)

Because the blood flow brings new endotoxin in contact with the membrane continuously and that the input endotoxin concentration will vary over time according to (1) changing seeding of endotoxin amount into the blood compartment and (2) a changing degradation by the immune system, it is easy to realize that no calculated prediction of how fast the membrane will be covered with endotoxin molecules can be formulated. Also, the endotoxin concentration in an in vivo system is low and can even approach the critical concentration where adsorbtion/disorption rates equal meaning that no netto adsorption longer takes place. Although the endotoxin concentration in blood is low a septic cascade that has started will continue, and the septic cascade is not directly proportional to the endotoxin concentration. The Langmuir equation is strictly valid on a static volume from which adsorption on a membrane takes place and not on a fluid flow. In addition, the Langmuir mathematics assumes that the endotoxin–poly-imine ethylene layer is monolayer, which need not be the case on the oXiris membrane [7].

All this sums up in a conclusion that an in vivo reality differs from a simplistic in vitro reality, but still the data from our 2 patients suggest that adsorption on the membrane is a strong actor in the endotoxin removal.

Statement of Ethics

Ethical permission was granted for this research by The Regional Ethics Board of Southern Sweden.

Disclosure Statement

M.E.B. and M.B. have got a research grant from Baxter for endotoxin research.

References

1 Rimmelé T, Kellum JA. Clinical review: blood purification for sepsis. Crit Care. 2011;15(1):205.
2 Cohen J. The immunopathogenesis of sepsis. Nature. 2002 Dec;420(6917):885–91.
3 Beutler B. Endotoxin, toll-like receptor 4, and the afferent limb of innate immunity. Curr Opin Microbiol. 2000 Feb;3(1):23–8.
4 Kellum JA, Kong L, Fink MP, Weissfeld LA, Yealy DM, Pinsky MR, et al.; GenIMS Investigators. Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. Arch Intern Med. 2007 Aug;167(15):1655–63.
5 Malard B, Lambert C, Kellum JA. In vitro comparison of the adsorption of inflammatory mediators by blood purification devices. Intensive Care Med Exp. 2018 May;6(1):12.
6 Yokota M, Kambayashi J, Tanaka T, Tsujinaka T, Sakon M, Mori T. A simple turbidimetric time assay of the endotoxin in plasma. J Biochem Biophys Methods. 1989 Mar;18(2):97–104.
7 Patiha, Heraldy E, Hidayat Y, Firdaus M. The langmuir isotherm adsorption equation: the monolayer approach. IOP Conf Ser: Mater Sci Eng. 2016; 107:012067.