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PLASMA ENDOTOXIN LEVEL OF HEALTHY DONORS

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The plasma level of endotoxin was determined in 116 healthy blood donors. After a routine physical and laboratory investigations the endotoxin level was determined with Limulus amebocyte lysate assay (LAL-test) by the chromogenic kinetic method of Bio-Whittaker Co. (USA). Its sensitivity was 0.005–50 EU/ml. The plasma level of endotoxin in most of the healthy donors was less than 1 EU/ml (in the range of 0.01–1.0 EU/ml), but always measurable. The average ±S.D. was 0.128±0.215 EU/ml. Because of the high standard deviation and high range of values, the data were distributed into two groups with the means of 0.05±0.022 EU/ml and 0.294±0.186 EU/ml. The difference between the groups was significant ($p<0.001$). In conclusion, endotoxin can be measured in plasma of healthy individuals.

Keywords: endotoxin in plasma, LAL-test

Introduction

It is well known that mammals – including human beings – have been living in symbiosis with Gram-negative intestinal bacteria for thousands of years. From the clinical point of view the question arises, whether endotoxin releasing from the cell wall of bacteria would enter the circulation of healthy individuals among physiological circumstances and what could be its consequences. Earlier observations have shown (Bertók and Mandel, unpublished experiments 1980) that a certain amount of endotoxin seems to be a must for the development of the immune system in germ free

pigs. However, few data have been published so far on the presence and concentration of endotoxin in the so-called “normal” plasma, i.e. in the plasma obtained from healthy people [5, 6, 15, 16, 18]. Based upon our current knowledge, we are not able to define either plasma endotoxin levels causing clinical effects, or the physiologic and pathophysiologic significance of endotoxin existing in the blood of healthy individuals.

Trying to find answers to these questions, we based our statements partly on the few articles available in this research field [5, 6, 15, 16], as well as on our present studies performed on healthy blood volunteers, as detailed below. Some recent observations reflecting upon the relationships between cytokines produced in pathological quantities (TNF-alpha, interleukins, etc.) and certain diseases (e.g. myocardial disorders) also highlight the problem.

Materials and methods

In order to get answer on the existence and levels of endotoxin in the blood of healthy people, 116 complaint-free, healthy blood-donors (59 male, 57 female) were chosen to this study. They were subjected to detailed anamnestic history and thorough physical examination followed by the laboratorial analysis of blood and urine samples taken in advance. The following parameters were determined using routine procedures: urinary protein and sugar excretion, blood-count, RBC sedimentation rate, Wassermann reaction, serum glutamate-piruvate transaminase (SGPT), HIV, serological examinations (HBsAg, HCV) with reference to hepatitis B and C virus. None of the 116 individuals showed any signs of pathognostic deviations.

Plasma endotoxin level was determined by Limulus amebocyte lysate assay (LAL-test) [10].

Today, only the last thrombin-like enzyme of the whole coagulation cascade and a synthetic peptide, attached to an aniline stain is used. As a result of the coagulation of the synthetic peptides the anilin stain is released and suitable for spectrophotometric determination at 405 nm. Due to the recent innovation in the methodology, the newly developed, computer assisted chromogenic-kinetic method follows the developing colour-reaction [12].

For determining the endotoxin in the plasma, blood samples were taken in the morning hours from the cubital vein of the fasting individuals into pyrogen-free tubes containing 3.8% sodium citrate anticoagulant (blood/citrate ratio 8:1). The plasma was separated from the cellular blood elements by centrifugation then followed by heat treatment (15 minutes at 80 $^{\circ}$C) and dilution of the sample (pyrogen-free distilled water used for 1:10 dilution) afterwards. The endotoxin level was determined by the above-mentioned chromogenic kinetic method using Bio-Whittaker reagents (Whittaker

Bioproducts, Walkersville, M.D., USA), Pyrosoft software system (Biomondex LTd., Budapest) and Twinreader apparatus (Labsystems, Finland). This way we could make examinations in the range of 0.005 and 50 EU/ml. 1 EU refers to 200 pg of standard endotoxin.

For statistical analysis data distribution curve evaluation studies and unpaired two-tailed Student's *t*-test were used.

Table I

Blood-plasma endotoxin level of healthy donors

Group	n	Endotoxin* EU/ml X, STD	Significance (p) (*t*-test)
1	88	0.05 0.022	< 0.005
2	26	0.294 0.186	

***** The endotoxin was determined by chromogenic kinetic LAL-method

Results

The average value of the test results was 0.128 EU/ml (25.6 pg/ml) ± 0.215. When examining healthy individuals other test-groups have shown similar results. Goto and his coworkers found the plasma endotoxin concentration of healthy old people at a 7±4 pg/ml level (which adequates 0.035 EU/ml) [16], while Novitsky et al. found the plasma endotoxin level of healthy adults at a 0.151±0.113 EU/ml level (which adequates 30,2 pg/ml) [17].

The experiments carried out resulted in a high range of values (0.014 EU/ml to 1.57 EU/ml. Due to this, the standard deviation (0.215) became higher than the average value of the test results (0.128), therefore we distributed the measured values into two groups with the means of 0.01–0.1 EU/ml and 0.1–1.0 EU/ml. We have found two extreme high endotoxin level values, exceeding the 1.0 EU/ml level. These values were not used for the discussion.

Most values – at 88 from 116 individuals tested – were under 0.1 EU/ml. This adequates to 76% of the tested people. At 22%, i.e. at 26 individuals 0.1–0.7 EU/ml values were found.

A significant difference was found between the average values of the two groups. Using statistical analyses a low standard deviation can be set for the two groups. When comparing the results of group 1 with group 2, a significant difference was found ($p<0.005$).

Regarding the blood endotoxin levels no difference was found between males and females.

Coherence between the age of patients and their plasma endotoxin level could not be proved.

Discussion

In the past few decades a significant level of knowledge accumulated on the significance and pathophysiological role of Gram-negative bacteria, as well as endotoxin (endotoxaemia) in serious infectious diseases often with fatal outcome [11]. On the other hand, endotoxin has already been proved not to be deleterious by all means, i.e. it has other than negative effects on biological systems, as well. It is rather probable that endotoxin is playing an important role in the ontogenesis of the developing lymphatic/immune system in higher level organisms living in natural surroundings.

Bertók and Mandel (not published data) have found that in germ-free animals the lymphatic system was seriously retarded (undeveloped) and the immune defense mechanisms were almost missing. Nevertheless, even within these conditions, the animals still retained the potential to respond to small quantities of endotoxin or detoxified endotoxin (TOLERIN®) featured by the forced development of these systems. This means that endotoxin can be considered as “stimulator” of the whole lymphatic/immune system.

Several data from the literature as well as our own earlier studies support the observations that certain diseases (inflammatory intestinal diseases, some liver and bile-duct diseases, different infections, sepsis, intestinal mucosa injury caused by radiation and cytotoxic drugs, haemodialysis-treatment for chronic renal insufficiency, etc.) are incident to the elevation of the plasma endotoxin level [2, 3, 14, 15, 19, 20]. According to some publications, the extent and, in certain cases, the duration of endotoxaemia was closely related to the severity and outcome of the illness. In these cases plasma endotoxin levels higher than 1.0 EU/ml were usual findings [4, 7, 8].

In accordance with others, our current results have also proved that some amount of endotoxin can be detected in the blood of each healthy individuals. As far as measurement ranges are considered, the results of the different research groups are in

rather good correlation [5, 6, 16, 18]. However, depending on the differences among *E. coli* strains, and the procedures used to extract and purify endotoxin that serves as standard for these measurements, some differences might occur in the specific activity (EU/pg).

Certain ideas and theories gained of endotoxin research, today can be regarded as proved. Nevertheless, questions always occurre that cannot even be answered with our present knowledge. The reason for complaint-free and asymptomatic behaviour at high endotoxin level [6] and *vica versa* for low plasma endotoxin level at severe diseases (e.g. in case of hepatic cirrhosis in advanced stage complicated with parenchymal and vascular decompensation), in which one could reasonably expect higher endotoxin levels, is still unclear [6]. Some research groups could not show any relationships between the endotoxin level and the severity of the clinical findings or the course of the disease [9, 16, 17]. Because of the low number of data available even worldwide, it has not been defined yet, what plasma endotoxin levels can be considered as still "physiological". Neither is declared, if endotoxin that is measurable in the blood plasma of healthy individuals has any physiological or pathophysiological significance. Even the pharmacokinetics of endotoxin is still missing, i.e. we do not know how fast plasma endotoxin level can change (turnover rate).

The significance of the above-mentioned becomes more outstanding if it is considered that – as it happened in our case – the plasma of even healthy blood donors may contain endotoxin at higher concentration. Consequently, it is questionable, whether endotoxin administered by blood is responsible for fever, shivering and eventually hypotension, evaluated as transfusion reaction. Based upon this finding it seems reasonable to determine endotoxin level along with the conventional parameters in the blood taken for transfusion purposes.

A possible reason for the yet uncleared questions is that even by using the best measuring methods, we have to face several problems. In the blood, there are factors that make the determination of endotoxin level harder. Certain molecules, e.g. HDL, haemoglobin, albumin, transferrin, anti-endotoxin antibodies, heparin, etc., are able to bind endotoxin at least partly [21]. Further on, it must also be considered that some compounds, substances, even some microenvironmental effects (actual pH values, viscosity of the sample on test, etc.) could influence the LAL-reaction (which is based on an enzymatic chain-reaction), resulting in the modification of the real values. Certain products of proteolysis mono- and divalent anions, cations, protease inhibitors, etc. are also belonging to these materials. On the other hand, there are factors in the blood that can activate the coagulation enzyme-system and result in pseudopositive outcomes. Newest endotoxin test methods – including the one that we used – try to

eliminate most of the interfering effects or take them into consideration when standardizing the method.

It is rather possible that the known but still undefined phenomenon called endotoxin tolerance, may play a role in the loose correlation between clinical findings and the actual endotoxin values. There are no exact data available on the rhythm and mechanisms how endotoxin is eliminated from the blood. It is rather assumable that after entering the blood stream endotoxin can be present just for a very short time period, for cells and groups of cells (hepatocytes, monocyte-macrophage system) that safeguard the organism, do their job by binding, uptaking, decomposing and excreting endotoxin rapidly. At the same time, "secondary" mediators (cytokines, TNF-alfa, nitrogen monoxide, etc.) released from the primary target cells of endotoxin can maintain the pathophysiological processes and now are blamed for the majority of the clinical manifestations.

It is likely that these facts are also playing a role in creating some "anomalies" that has not been satisfyingly cleared yet. Alltogether, there are few problems that have to be definitely taken into consideration when evaluating our own results, as well as those found in the literature.

References

1. Bertók,L.: Stimulation of nonspecific resistance by radio-detoxified endotoxin. In: Friedman,H., Klein,T.W., Nakano,M., Nowothny,A. (eds): Endotoxin. Plenum Publishing Corporation 1990, 677–680
2. Bertók,L.Jr., Sztanyik,L.B., Bertók,L.: Possible role of bile deficiency in the development of intestinal syndrome of acute radiation disease. Acta Phys Hung **79**, 29–32 (1992)
3. Bode,C.: Endotoxemia in patients with alcoholic and non-alcoholic cirrhosis and in subjects with no evidence of chronic liver disease following acute alcoholic excess. J Hepatol **4**, 8–14 (1987)
4. Brandtzaeg,P., Kierulf,P., Gaustad,P.: Plasma endotoxin as a predictor of multiple organ failure and death in systemic meningococcal disease. J Infect Dis **159**, 195–202 (1989)
5. Friberger,P.: The design of a reliable endotoxin test. Prog Clin Biol Red **189**, 139–149 (1985)
6. Goto,T., Edén,S., Nordenstam,G.: Endotoxin levels in Sera of Elderly Individuals. Clinical and Diagnostic Laboratory Immunol **1**, 684–688 (1994)
7. Hurley,J.C.: Concordance of endotoxemia with Gram-negative bacteremia in patients with Gram-negative sepsis: A meta-analysis. J Clin Microbiol **32**, 2120–2127 (1994)
8. Hurley,J.C.: Endotoxemia: Methods of detection and clinical correlates. Clin Microbiol Rev **8**, 268–292 (1995)
9. Ketchum,P.A., Parsonnet,J., Stotts,L.S.: Utilization of a chromogenic Limulus amebocye lysate blood assay in a multi-center study of sepsis. J Endotoxin Res **4**, 9–16 (1997)

10. Levin,J., Tomasulo,P.A., Oser,R.S.: Detection of endotoxin in human blood and demonstration of an inhibitor. J Lab Clin Med **75**, 903–911 (1970)
11. Levin,J., Poore,T.E., Zauber,N.P.: Detection of endotoxin in the blood in patients with sepsis due to Gram negative bacteria. N Engl J Med **283**, 1313–1316 (1970)
12. Lindsay,G.K., Roslansky,P.F., Novitsky,T.J.: Single-step, chromogenic Limulus amebocyte lysate assay for endotoxin. J Clin Microbiol **27**, 947–951 (1989)
13. Nádházi,Z., Takáts,A., Offenmüller,K.: Endotoxin (LPS) in the blood plasma. International Congress of Stress. 1997 Abstract, Budapest, Hungary
14. Nisbeth,U., Hallgren,R., Eriksson,Ö.: Endotoxemia in chronic renal failure. Nephron **45**, 93–97 (1987)
15. Nitsche,D., Kriewitz,M., Rossberg,A.: The quantitative determination of endotoxin in plasma samples of septic patients with peritonitis using the chromogenic substrate and its correlation with the clinical course of peritonitis. Prog Clin Biol Res **231**, 417–429 (1987)
16. Novitsky,T.J.: Limulus amebocyte lysate (LAL) detection of endotoxin in human blood. Journal of Endotoxin Res **1**, 253–263 (1994)
17. Novitsky,T.J.: Endotoxin: Does its detection provide diagnostic benefit? Intensive Care Med **20**, S102 (1994)
18. Offenmüller,K., Takáts,A., Nádházi,Z.: Quantitative determination of endotoxin in the blood plasma using kinetic chromogen Limulus test. Lab Diagn **XX**, 165 (1993)
19. Pearson,F.C., Dubczak,J., Weary,M.: Detection of endotoxin in the plasma of patients with Gram-negative bacterial sepsis by the Limulus amoebocyte lysate assay. J Clin Microbiol **21**, 865–868 (1985)
20. Ren-Shin,L., Fa-Yauh,L., Shou-Dong,L.: Endotoxemia in patients with chronic liver disease: relationship to severity of liver diseases, presence of esophageal varices and hyperdynamic circulation. J Hepatol **22**, 165–172 (1995)
21. Wellman,W., Fink,P.X., Benner,F.: Endotoxemia in active Crohn's disease. Treatment with whole gut irrigation and 5-aminosalicylic. Gut **27**, 814–820 (1986)