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High serum levels of fatty acid–binding protein 7 in diabetic rats with experimental sepsis

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Abstract

Sepsis is a disease that affects a wide variety of individuals, including the young, the elderly, and those admitted to the hospital with diverse acute or chronic conditions. Because sepsis is such a heterogeneous disease, some researchers believe that personalized medicine may represent a promising means of improving the prognosis for certain patients. Of those who develop sepsis, diabetic patients remain a significant proportion, because diabetes is a metabolic disorder that is associated with disturbances in the immune system, which facilitates bacterial infections. Fatty acid–binding proteins (FABPs) are a family of transport proteins with an important role in metabolism; therefore, we decided to measure their levels in diabetic rats, as part of a search for a novel biomarker of sepsis. Diabetes was experimentally induced in male Wistar rats, some of which then underwent cecal ligation and puncture, and the levels of FABP4 and FABP7 were measured in their serum and key tissues. Serum FABP7 levels in diabetic septic rats were significantly higher than those in non-diabetic septic rats. Consequently, we propose that FABP7 should be further investigated as a potential biomarker of sepsis in diabetic patients.

Keywords

biomarker, diabetes, FABP7, inflammation, sepsis

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Introduction

Sepsis is a critical condition that is associated with prolonged hospital stays and a high mortality rate. It is characterized by an inappropriate systemic inflammatory response to microorganisms that invade the bloodstream, frequently leading to organ dysfunction and death.[1]

The frequency of sepsis is greater at the extremes of age and in patients with other clinical or surgical conditions.[2] Because sepsis develops in such a heterogeneous population, some authors have argued that personalized medicine represents a promising means of improving patient care in this critical situation.[3,4] Indeed, in the future, patients with widely differing clinical conditions, such as the elderly, the diabetic, or the obese, may benefit from different approaches to the diagnosis and treatment of sepsis.

Diabetes mellitus (DM) is one of the largest global epidemics of the 21st century,[5] is a known risk factor for infection, and is present in ~20% of septic patients.[6] Patients with type 1 and type 2 DM display impairments in innate and adaptive

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immune responses,[7,8] and a complex interplay between metabolic and immune pathways is thought to be important in the pathogenesis of these diseases.[9]

Fatty acid–binding proteins (FABPs) comprise a family of lipid transporters that are crucial mediators of metabolism and other biological processes which support systemic immunometabolic networks and maintain homeostasis, as has been extensively reviewed in recent publications.[10] However, the role of FABPs in sepsis remains obscure, despite their numerous other effects on metabolism and the regulation of immunity, and the numerous types of cross-talk that they mediate between these systems.

Some years ago, our group showed that the serum levels of FABP6, a protein produced by enterocytes, are higher during septic shock than those in healthy donors.[11] Here, we measured the protein and mRNA levels of FABP4 and FABP7 in a model of experimental sepsis and compared these parameters between diabetic and non-diabetic septic animals.

Materials and methods

Animals

Male Wistar rats that were 8 weeks old weighing 200–350 g were used in this study. The animals were housed under climate-controlled conditions (22°C ± 1°C) on a 12:12-h photoperiod and were provided with food and water ad libitum. The experiments were performed in the Emergency Medicine Department Laboratory of the University of Sao Paulo, Brazil. The protocol was approved by the Ethics Committee for Animal Use of the Faculty of Medicine, University of Sao Paulo (CEUA—FMUSP, authorization #140/14), in accordance with the principles of the National Council for the Control of Animal Experimentation (Concea).

Induction of diabetes using alloxan

Alloxan-induced injury of pancreatic beta cells was used to induce type I diabetes in the animals. Before induction, the rats were weighed and their blood glucose levels were measured (OneTouch Ultra Blood Monitor; Johnson & Johnson, New Brunswick, New Jersey, USA). Animals were then anesthetized by isoflurane inhalation and were given a single dose of alloxan (Sigma-Aldrich, Inc., St Louis, MO, USA) of 42 mg/kg through the penile vein. Glycemia was measured after 10 days and the animals with blood glucose > 200 mg/dL were considered to be diabetic.

Induction of sepsis using cecal ligation and puncture

We induced fecal peritonitis in rats using cecal ligation and puncture (CLP) as described previously.[12] Briefly, the animals were anesthetized using 80 mg/kg ketamine (Parke-Davis, Morris Plains, New Jersey, USA) and 10 mg/kg xylazine (Bayer, Leverkusen, Germany), and the cecum was ligated and punctured twice with a 21G needle, allowing the release of fecal material into the peritoneal cavity, eventually leading to systemic infection.

Mortality curve

To obtain a mortality curve, 10 healthy rats and 10 diabetic rats underwent CLP. The rats were observed every 12 h until death or euthanasia on day 5.

Quantitative real-time polymerase chain reaction

The animals were divided into four groups (healthy control, diabetic control, septic, and diabetic septic; n = 6/group). Serum and tissue samples (leukocytes, adipose tissue, skeletal muscle, and liver) were collected from controls 8 h after CLP and stored at −80°C. Adipose tissue was obtained from around the epididymis and muscle samples from the gastrocnemius muscle. FABP4 and FABP7 gene expression was quantified in tissue samples as described below.

Total RNA was extracted with TRIzol (Invitrogen, USA), according to the manufacturer's guidelines, and was treated with DNase I (Invitrogen, Carlsbad, California, USA).

Real-time polymerase chain reaction (PCR) was performed using SuperScript Platinum III One-Step kits containing SYBR Green (#11736-051; Invitrogen). One-Step production and amplification of cDNA was performed on a StepOne thermocycler (Applied Biosystems, Carlsbad, CA, USA), and the products were identified on a 1.5% agarose gel (100 ng total RNA per sample).

Relative expression levels were evaluated by the $2^{-\Delta\Delta CT}$ method, using the reference gene β-2 microglobulin (β2M) to normalize the target gene expression data, which is expressed in arbitrary units.

Protein measurements

FABP4 and FABP7 levels were quantified in the serum by enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions (MyBioSource, San Diego, CA, USA).

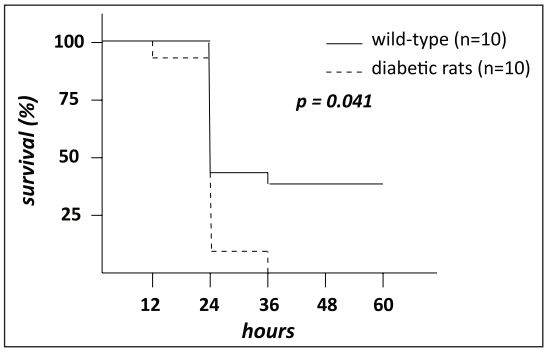

Figure 1. Mortality curve comparing healthy and diabetic rats that underwent cecal ligation and puncture (n = 10/group).

Statistical analysis

The Kaplan–Meier and log-rank methods were used to analyze mortality. Continuous variables were analyzed by analysis of variance (ANOVA). Post hoc analysis was performed using the Mann–Whitney *U* test. Results are reported as mean ± standard deviation. $P \leq 0.05$ was considered to be statistically significant.

Results

We found that the diabetic rats exhibit greater mortality than the previously healthy rats when subjected to CLP (Figure 1).

All the study groups exhibited similar expression of FABP4 in their leukocytes and liver (Figure 2(a) and (b)), and there were also no differences in serum FABP4 levels among the study groups (Figure 3). However, FABP4 gene expression levels in adipose tissue and muscle were significantly higher in the previously healthy rats than in the diabetic rats following CLP (Figure 2(c) and (d)).

In our study, no differences were found in the expression of FABP7 among the study groups in the tissues investigated (Figure 4). Low levels of FABP7 mRNA were detected in the livers of these

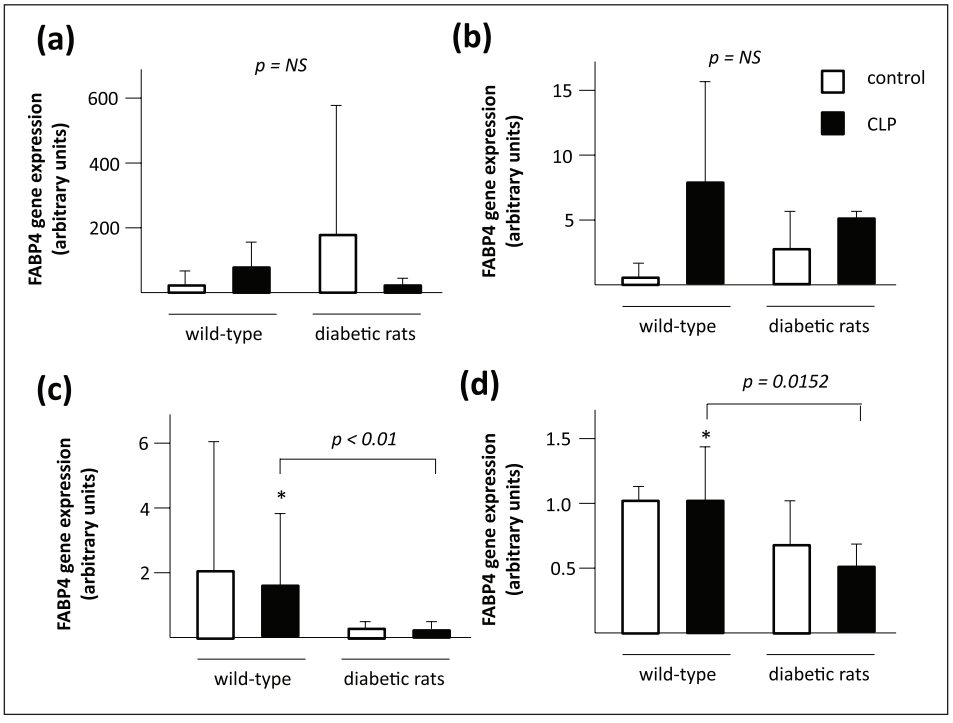

Figure 2. FABP4 gene expression in the (a) blood, (b) liver, (c) adipose tissue, and (d) muscle of healthy and diabetic Wistar rats that did or did not undergo cecal ligation and puncture (n = 6/group).

animals (Figure 4(b)), but FABP7 mRNA was detected in the leukocytes of the septic diabetic rats (Figure 4(a)). However, serum FABP7 levels were significantly higher in diabetic rats subjected to CLP than those in the other study groups (Figure 5).

Discussion

Despite extensive basic research and numerous clinical trials in the recent decades, advances in the treatment of sepsis have been very disappointing.[13] Because patients present with diverse clinical conditions and comorbidities and there are multiple sources of infection and etiologic agents, it is not surprising that a "magic bullet" has never been found. Some researchers therefore contend that patient care should be individualized[14] because the factors such as age, source of infection, bacterial agent, and the presence of comorbidities shape the immune and inflammatory responses in sepsis, leading to a variety of outcomes.[15]

Early diagnosis of sepsis is crucial because it permits timely treatment and better prognosis. Intensive research by the scientific community has been undertaken to identify biomarkers of early infection,[16,17] because it is difficult for the physician to differentiate many non-infectious inflammatory syndromes, such as acute pancreatitis, burns, multiple trauma, and major surgeries, from sepsis using clinical signs and blood culture alone.[18,19] However, a reliable biomarker of sepsis has not been identified to date. We believe that, in the future, single or a combination of biomarkers will be used based on the patient profile. Thus, patients must be classified and candidate biomarkers interrogated in specific patient sub-populations. Hyperglycemia is a common finding in septic patients;[20] therefore, we decided to attempt to identify a biomarker of sepsis connected to this.

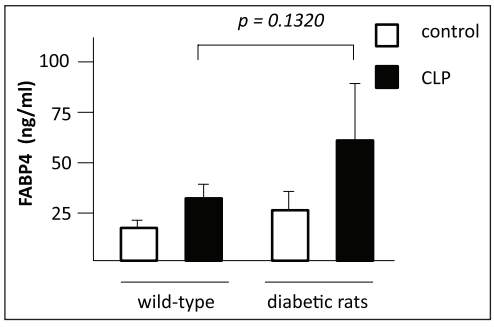

Figure 3. Serum levels of FABP4 in healthy and diabetic Wistar rats that did or did not undergo cecal ligation and puncture (n = 6/group).

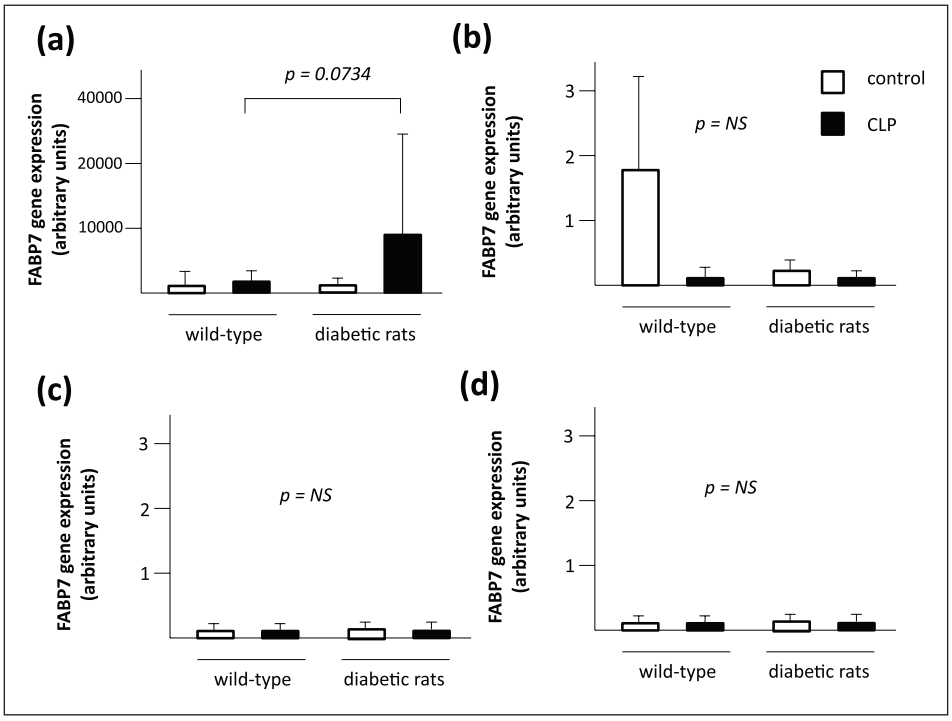

Figure 4. FABP7 gene expression levels in the (a) blood, (b) liver, (c) adipose tissue, and (d) muscle of healthy and diabetic Wistar rats that did or did not undergo cecal ligation and puncture (n = 6/group).

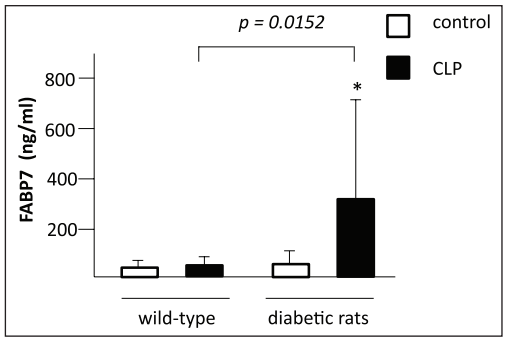

Figure 5. Serum levels of FABP7 in healthy and diabetic Wistar rats that did or did not undergo cecal ligation and puncture (n = 6/group).

Fatty acids serve many biological functions within the cell, such as acting as energy sources, components of cell membranes, and signaling molecules. FABPs are a conserved family of transporters that bind these ligands and other lipids with high affinity and transport them between diverse intracellular or extracellular environments.[21]

FABP4, also known as adipocyte FABP (A-FABP) or aP2, is mainly expressed in adipocytes, macrophages, and dendritic cells.[22] In general, the presence of FABPs in the circulation is considered to be a marker of tissue injury,[11] but FABP4 is secreted from adipocytes under certain conditions, such as during the development of metabolic syndrome or cardiovascular diseases, when it can act as an adipokine.[22]

Interestingly, a recent publication by Hu et al. described the induction of FABP4 in the liver during sepsis. Moreover, pharmacological inhibition of FABP4 improved the survival of mice subjected to CLP.[23] In our study, all of the treatment groups exhibited similar expression of FABP4 in leukocytes and in the liver, and there were no differences in serum FABP4 among the study groups. However, FABP4 expression levels in adipose tissue and muscle were significantly higher in previously healthy rats than in diabetic rats subjected to CLP. We suspect that this is due to immunosuppression in diabetic animals succumbing to infection, but further studies will be necessary to explain this finding.

FABP7, also known as brain FABP (B-FABP), is expressed in glial cells and neurons. It is thought to play an essential role in the development of the brain[24,25] and in many brain disorders.[26,27] In addition, FABP7 has been detected in Kupffer cells[28] and in certain types of cancer.[29–32] Interestingly, FABP7 regulates phagocytosis and cytokine production in Kupffer cells during liver injury.[33]

The higher serum levels of FABP7 observed in septic diabetic rats suggest the presence of more severe tissue injury in this group. We hypothesize that FABP7 is being released from the brain under these circumstances, because low levels of FABP7 mRNA were detected in the liver of these animals. However, FABP7 mRNA was also detected in the leukocytes of septic diabetic rats. Because leukocytes do not normally produce FABP7, these data imply that the FABP7 pathway may be deregulated in several tissues, but further studies are necessary to investigate this possibility.

The purpose of this study was to attempt to identify a reliable biomarker of sepsis to be used in the diabetic population. Serum FABP7 was higher in diabetic septic rats than in the other study groups, making FABP7 an interesting candidate for further investigation as a potential biomarker of sepsis in diabetic patients, which could be used in the context of personalized medicine.

DM is a metabolic disease that affects the immune response and facilitates infection. Here, using a rodent model, we have identified FABP7 as a potential serum biomarker of severe infection in the presence of hyperglycemia. Other studies will be necessary to confirm this finding in humans, as well as to investigate the role of FABPs in this clinical scenario.

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