Journal of Diabetes and Its Complications 28 (2014) 844–849

Contents lists available at ScienceDirect

Journal of Diabetes and Its Complications

journal homepage: WWW.JDCJOURNAL.COM

Hyperglycemia is associated with lower levels of urokinase-type plasminogen activator and urokinase-type plasminogen activator receptor in wound fluid

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ARTICLE INFO

Article history:
Received 18 February 2014
Received in revised form 21 July 2014
Accepted 31 July 2014
Available online 7 August 2014

Keywords:
Wound healing
Hyperglycemia
Plasminogen activation
Wound fluid
Diabetes

ABSTRACT

Aims: Wounds in patients with hyperglycemia show impaired healing. Plasminogen activation is crucial in several overlapping phases of wound healing process. In this study, we aimed i) to compare acute wound fluid in patients with hyperglycemia and normoglycemia, ii) to focus on the elements of plasminogen activation in the wound fluid, and iii) to determine if the acute wound fluid characteristics are associated with surgical site infections.

Methods: In a cohort of 54 patients, a closed suction drain was placed in the wound above the anterior abdominal wall fascia under the skin in order to collect postoperative acute wound fluid samples for 3 following days after colorectal surgery. Patients were classified as normoglycemic ($n = 25$) or hyperglycemic ($n = 29$; 17 with type 2 diabetes and 12 with stress induced hyperglycemia). Surgical site infection was defined according to the Centers for Disease Control criteria. The levels of urokinase-type plasminogen activator (uPA), urokinase-type plasminogen activator receptor (uPAr), plasminogen activator inhibitor-1 (PAI-1), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and fibroblast growth factor-1 (FGF-1) were measured in the wound fluid.

Results: Compared to normoglycemic subjects, patients with hyperglycemia had significantly lower levels of uPA and uPAr in the wound fluid despite similar or even higher circulating levels. There was no significant difference in IL-1β, TNF-α, PAI-1 and FGF-1 levels. In the whole study population, the wound fluid levels of uPA and uPAr were negatively correlated with circulating glucose levels. No difference was detected in the wound fluid characteristics of patients with and without surgical site infection.

Conclusion: Patients with hyperglycemia exhibit decreased levels of uPA and uPAr in the wound fluid, suggesting a local failure in plasminogen activation at the wound site.

1. Introduction

Wound healing is a complex process which involves several overlapping stages that includes hemostasis, inflammation, granulation tissue formation, reepithelialization and tissue remodeling (Barrientos, Stojadinovic, Golinko, Brem, & Tomic-Canic, 2008; Hanson, Langemo, Thompson, Anderson, & Hunter, 2005; Toriseva & Kahari, 2009). Activation of plasminogen (Plg) plays an important role in proteolytic degradation of extracellular matrices during the wound healing process (Botta et al., 2012). It has been shown that wound healing is severely impaired in mice made deficient in Plg (Romer et al., 1996). In Plg −/− mice, poor wound healing was associated with delayed removal of necrotic debris, reduced leucocyte infiltration and smooth muscle cell accumulation and migration, and decreased neointima formation (Carmeliet, Moons, Ploplis, Plow, & Collen, 1997).

Wound healing is altered in diabetes for several potential reasons. Inflammatory phase of wound healing has been shown to be prolonged in patients with diabetes as a result of glycosylation that impairs neutrophil and macrophage functions, especially in patients

Abbreviations: ADA, American Diabetes Association; BMI, Body mass index; CDC, Centers for Diseases Control; DISSI, Deep incisional surgical site infection; FGF-1, Fibroblast growth factor-1; IL-1β, Interleukin-1β; Plg, Plasminogen; PAI-1, Plasminogen activator inhibitor-1; SISSI, Superficial incisional surgical site infection; tPA, Tissue-type plasminogen activator; TNF-α, Tumor necrosis factor-α; uPA, Urokinase-type plasminogen activator; uPAr, Urokinase-type plasminogen activator receptor.

Disclosure: None.

Conflict of interest: The authors declare that they have no competing financial interests.

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http://dx.doi.org/10.1016/j.jdiacomp.2014.07.013
1056-8727/

with poor glycemic control (Fahey et al., 1991; Lecube, Pachon, Petriz, Hernandez, & Simo, 2012). It has been proposed that diabetes is associated with less pliable erythrocytes which are associated with impaired oxygen transportation to the wound for tissue metabolism (Oughton & Barnes, 1981). Studies have reported that the number of endothelial progenitor cells, which derive from bone marrow, normally travels to the sites of injury and are essential for the formation of blood vessels and the wound healing, is decreased in the circulation and at wound sites in diabetes (Cianfarani et al., 2013; Saito, Yamamoto, & Yamamoto, 2012). In addition, diabetes has been shown to play a significant predisposing role for surgical site infections in many surgical disciplines (Harrop et al., 2012).

The success of the wound healing requires that all the players of the process work in a very well organized and coordinated fashion. The impact of systemic markers on the assessment of the wound healing is questionable as they may more likely reflect the sum of different effects on all over the body. At this point, the wound fluid turns out to be an easily accessible way to investigate the local wound microenvironment. Alterations in the wound fluid proteins, cytokines or growth factors are most likely to give evidence for describing the local wound. The wound fluid composition is modified by factors which are secreted by local cells in the wound or by cells that migrate into the wound. Since the cellular features are constantly changing during acute wound healing, the wound fluid would also give information regarding wound characteristics over time.

In this current study, we aimed at comparing the wound fluid characteristics of patients with and without hyperglycemia. We specially focused on the elements of Plg activation in the wound fluid. We also attempted to determine if any of these parameters detected in the acute wound fluid is associated with the development of surgical site infections. In addition to the elements of Plg activation, we chose to measure the levels of tumor necrosis factor-α (TNF-α), interleukin-1 β (IL-1β) and fibroblast growth factor-1 (FGF-1) in the wound fluid. As mentioned above, the wound healing has several overlapping stages. Neutrophils and macrophages are the key players of inflammation stage. They produce TNF-α and IL-1β that will recruit fibroblasts and epithelial cells at the wound site. It has been shown that the levels of the proinflammatory cytokines, TNF-α and IL-1β, are higher in the nonhealing wounds compared to the healing wounds (Trengove, Bielefeldt-Ohmann, & Stacey, 2000). Growth factors are other soluble factors found in the wound fluid. Macrophages that emerged from monocytes express several growth factors. FGF-1 is one of them that is produced by macrophages, endothelial cells and fibroblasts, and promotes angiogenesis, endothelial cell activation, keratinocyte proliferation and migration, and extracellular matrix deposition (Lobmann, Schultz, & Lehnert, 2005).

2. Materials and methods

2.1. Study population

Postoperative acute wound fluid samples were collected consecutively after colorectal surgery in a cohort of 54 adult patients. Among them, 13 patients had already been diagnosed with type 2 diabetes. In 41 patients without previous evidence of diabetes, blood glucose levels were monitored before, during and after the surgery. Patients with newly detected hyperglycemia were considered having type 2 diabetes if HbA1c level was greater than 6.5%. Others were tested using a 75 g oral glucose tolerance test (OGTT) at least six months after the recovery from the colorectal surgery. Type 2 diabetes was defined according to the American Diabetes Association's (ADA) recommendations (anon, 2013). Among patients with newly detected hyperglycemia (n = 16), four patients were classified as type 2 diabetes after the postsurgery follow-up. The patients with transient hyperglycemia (with a fasting glucose ≥ 126 mgl/dL or random glucose ≥ 200 mg/dL) during the hospitalization that reverted to normal range after discharge (diabetes ruled out by HbA1c and 75 g OGTT) were classified as stress induced hyperglycemia group (n = 12). Twenty-five patients without hyperglycemia were stratified in the normoglycemic group.

The study was approved by the local research ethics committee of the Dokuz Eylul University. Informed consent was obtained in all cases.

2.2. Operation procedure

All patients were prepared for surgery by overnight fasting, and benzodiazepines were used for premedication. Mechanical bowel preparation was made by phosphosoda. The skin was shaved immediately before surgery and prepared by using povidone-iodine. The surgery was performed under general anesthesia. All patients had intravenous antimicrobial prophylaxis at the anesthesia induction time with cefuroxime axetil 1.5 g and metronidazole 500 mg. At the end of operation, no wound analgesia was applied. The patients with hyperglycemia were treated with intravenous insulin intraoperatively to maintain glycemic control. Human regular insulin was used. Target blood glucose level was 100–180 mg/dl. Postoperatively, the patients were maintained on insulin when necessary.

2.3. Collection of wound fluid

In addition to the routine intraperitoneal drain, an extra closed suction drain (Eczacibasi, Istanbul, Turkey) was placed in the wound above the anterior abdominal wall fascia under the skin in order to monitor local wound microenvironment. The surgical wound fluid was collected in a closed sterile collection bag. The wound fluid was collected on postoperative days 1–3 at 24-hour intervals. The wound drain was removed on postoperative day 4, and the patients were discharged in ten days after the surgery. Cultures were taken from the blood and wound drainage fluid just before removing the wound drain.

2.4. Definition of wound infection

All patients were examined for wound infections postoperatively. The wound infections were categorized according to the 1999 Guideline for Prevention of Surgical Site Infection of Centers for Diseases Control (CDC) (Mangram, Horan, Pearson, Silver, & Jarvis, 1999). Superficial incisional surgical site infection (SISSI) was defined as an infection occurring within 30 days after the operation and infection involving only skin or subcutaneously tissue of the incision. Deep incisional surgical site infection (DISSI) was defined within 30 days after the operation if no implant is left in place or within 1 year if implant is in place and the infection appears to be related to the operation and infection involves deep soft tissues (e.g. fascial and muscle layers) of the incision.

2.5. Laboratory methods

The wound fluid was put in a sterile tube without additives, centrifuged within the next 15 minutes at 3000 g for 15 minutes (Nuve, Ankara, Turkey), and the supernatant was separated and stored in sterile plastic tubes at −80 °C until analysis. A plasma sample was also taken at day 1. Blood was taken from the cannulated antecubital vein between 8:00 a.m. and 10:00 a.m. Blood samples were transferred into the tubes containing buffered citrate for coagulation proteins and tubes suitable for serum separation. Tubes were centrifuged at 3000 g for 10 min. Serum and plasma were extracted, aliquoted and stored at −80 °C until analysis. Plasma glucose levels were measured by a colorimetric method with the Roche/Hitachi D/P Modular System Autoanalyzer (Roche Diagnostics, Basel, Switzerland). Capillary blood glucose levels were analyzed on a glucometer (Abbott, IL, USA) by finger stick. Both the wound fluid and blood samples were analyzed for IL-1β, TNF-α, FGF-1, urokinase-type