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ORIGINAL ARTICLE

Upregulation of the proinflammatory cytokine-induced neutrophil chemoattractant-1 and monocyte chemoattractant protein-1 in rats' intestinal anastomotic wound healing—Does it matter?

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Summary *Background:* The proinflammatory cytokines and growth-promoting factor are essential components of the wound healing process. We hypothesized that under healthy conditions, faster healing of intestinal anastomotic wound is due to an early upregulation of proinflammatory cytokines, cytokine-induced neutrophil chemoattractant-1 (CINC-1) that is followed by a quicker upregulation of homeostatic chemokine, monocyte chemoattractant protein-1 (MCP-1) and late upregulation of transforming growth factor (TGF-β).
Methods: We characterized the time course of CINC-1, MCP-1 and TGF-β release at four wounds (skin, muscle, small bowel, and colonic anastomosis) after surgery on 38 juvenile male Sprague Dawley rats. The tissue samples of each site were harvested at 0 (control), 1, 3, 5, 7 and 14 days postoperatively ($n = 6–8$/group) and analyzed by ELISA kits for CINC-1, MCP-1 and TGF-β.
Results: CINC-1 expression peaked earlier in muscle and colonic wounds when compared to skin and small bowel. MCP-1 levels were elevated early in skin and muscle wounds, but later expression of MCP-1 was shown in colonic wounds. TGF-β levels were unchanged in all wound sites.

Conflicts of interest: The authors declare that they have no financial or non-financial conflicts of interest related to the subject matter or materials discussed in the manuscript.

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Conclusion: An earlier peak in CINC-1 levels and later expression of MCP-1 were seen in colonic wounds, but no significant increase in TGF-β levels was observed. These findings support the early healing process in intestinal anastomotic wounds.

1. Introduction

The most common complication following colorectal surgery is anastomotic leakage, which is associated with a high mortality rate.[1,2] Wound healing is a complex procedure that requires a highly regulated series of events, including interactions between many cell types, production of different soluble factors, and growth of matrix components. Normal wound healing has four phases—hemostasis, inflammation, tissue formation, and tissue remodeling.[3] The primary goal of wound repair is to achieve hemostasis, which represents the first step in wound healing.

The inflammatory phase begins after 6 hours of tissue injury. This phase is associated with some important events, such as migration of activated neutrophils, macrophages, and lymphocytes from the circulation into the injured tissue. Neutrophils are the first leukocytes to infiltrate the injured tissue, followed by monocytes.[4] Engelhardt et al[5] reported that neutrophil infiltration into a human skin wound is extremely high during the initial days, reaching a maximum level on Day 1. Neutrophils are dominant in the wound site during the initial days. By contrast, macrophages (activated monocytes) reached their maximum level on Day 2. Chemokines selectively mediate the recruitment of neutrophils and monocytes in the affected area. Interleukin-8 (IL-8) mRNA expression in the wound site is maximum on Day 1,[6] and then it declines to reach its lowest level on Day 4. Another neutrophil chemokine, growth-regulated oncogene (GRO-α), is also expressed within 4 days after wounding. There is a strong correlation between the levels of IL-8, GRO-α, and neutrophil infiltration. The cooperative expression of IL-8 and GRO-α in the wound site enhances neutrophil migration to this site because each of them stimulates neutrophils via different receptors. Importantly, rats lack a homolog of human IL-8, but they express a cytokine with similar properties and as potent a chemoattractant as IL-8, named cytokine-induced neutrophil chemoattractant (CINC-1).[7–9]

Monocytes infiltrate the injured tissue in response to chemotactic cytokines such as monocyte chemoattractant protein-1 (MCP-1). MCP-1 is released from basal keratinocytes adjacent to the epidermis or from endothelial cells.[6] Peak levels of MCP-1 are detected 1—2 days after skin wounding and slowly decline thereafter to Day 7.[5] Transforming growth factor-α (TGF-α) is another important cytokine for extracellular matrix synthesis and remodeling, which is released by monocytes and macrophages. These growth factors and cytokines function to promote the last two phases of healing: tissue formation and tissue remodeling.[10] Thus, an alteration of the expression or function of inflammatory cytokines may lead to the persistence of an inflammatory phase and alter the normal wound healing process. Therefore, successful acute wound healing occurs when proinflammatory cytokines, such as CINC-1, control the recruitment of neutrophils in inflammatory and tissue injury phases, and homeostatic chemokines, such as MCP-1, fulfill the housekeeping functions in cleaning the wound from debris during the hemostasis phase.[11]

Based on these observations, we hypothesized that under healthy conditions, faster healing of intestinal anastomotic wound in comparison to skin and muscle incisional wound is due to early upregulation of proinflammatory cytokine (CINC-1), followed by quicker upregulation of homeostatic chemokine (MCP-1) and late upregulation of TGF-β. To the best of our knowledge, this is the first study to investigate temporal expressions of CINC-1 and MCP-1 at four different tissues in rats: skin, muscle, small intestine, and colon.

2. Materials and methods

2.1. Animals

Thirty-eight male Sprague—Dawley rats (200—250 g) (animals were obtained from animal house of College of Medicine, King Saud University) were used for the study. Each rat was housed individually in a single cage and placed in a room with controlled temperature and light/dark cycle (12 h/12 h). The animals were allowed *ad libitum* intake of normal rat pellet. Thirty-eight animals, divided into groups with six rats in each group, underwent surgery; enzyme-linked immunosorbent assay (ELISA) was performed for CINC-1, MCP-1, and TGF-β at the following time points: 0 (control), Day 1, Day 3, Day 5, Day 7, and Day 14. Studies were conducted according to a protocol approved by the Animal Care Committee of the College of Medicine, King Saud University, Riyadh, Saudi Arabia.

2.2. Surgical procedure

The animals were anesthetized with 1—2% inhalational halothane. A single preoperative prophylactic dose of cefazolin (30 mg/kg; Novopharm Limited, Toronto, Ontario, Canada) was administered subcutaneously. The abdomen was prepared, shaved, and entered through a midline incision. Transection with immediate reanastomosis was performed both at the terminal ileum (10 cm proximal to the ileocecal valve) and at the transverse colon (1 cm proximal to the splenic flexure). The techniques were similar to previously published methods.[12,13] A standardized end-to-end anastomosis was performed with 8—10 interrupted inverting sutures using 6-0 monofilament polypropylene (Prolene TM; Ethicon, Parsippany, NJ, USA). Polypropylene was chosen because of its inert characteristics, minimizing the inflammatory reaction associated with resorption of an