Video Article

Collecting and Measuring Nociceptive and Inflammatory Mediators in Surgical Wounds

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Abstract

We describe a methodology by which we are able to collect and measure inflammatory and nociceptive biochemical mediators at the surgical wound site. Collecting site-specific biochemical markers allows us to evaluate the relationship between surgical wound and serum levels; determine any associations between mediator release, pain and analgesic consumption; and evaluate the effect of systemic and peripheral drug administration on surgical wound biochemistry.

This methodology has been applied to healthy women undergoing elective cesarean delivery with spinal anesthesia. Wound exudate and serum mediators, in conjunction with pain scores and analgesics consumption were measured at 1, 6, 24, and 48 hours post-cesarean delivery. Biochemical mediators that were detected included IL-1□, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, TNF□, INF□, G-CSF, GM-CSF, MCP-1 and MIP-1□, nerve growth factor (NGF), prostaglandin E2 (PG-E2) and substance P. We found no correlations between wound and serum cytokines concentrations or time-release profiles (J Pain. 2008 Jul 9(7):650-7). This article describes and demonstrates the feasibility of collecting and assaying nociceptive and inflammatory mediators in surgical wounds at specific time points. The lack of significant correlations between serum and wound levels shows the importance of determining site-specific release if surgical wounds and localized pathologies are to be studied.

Video Link

The video component of this article can be found at http://www.jove.com/video/962/

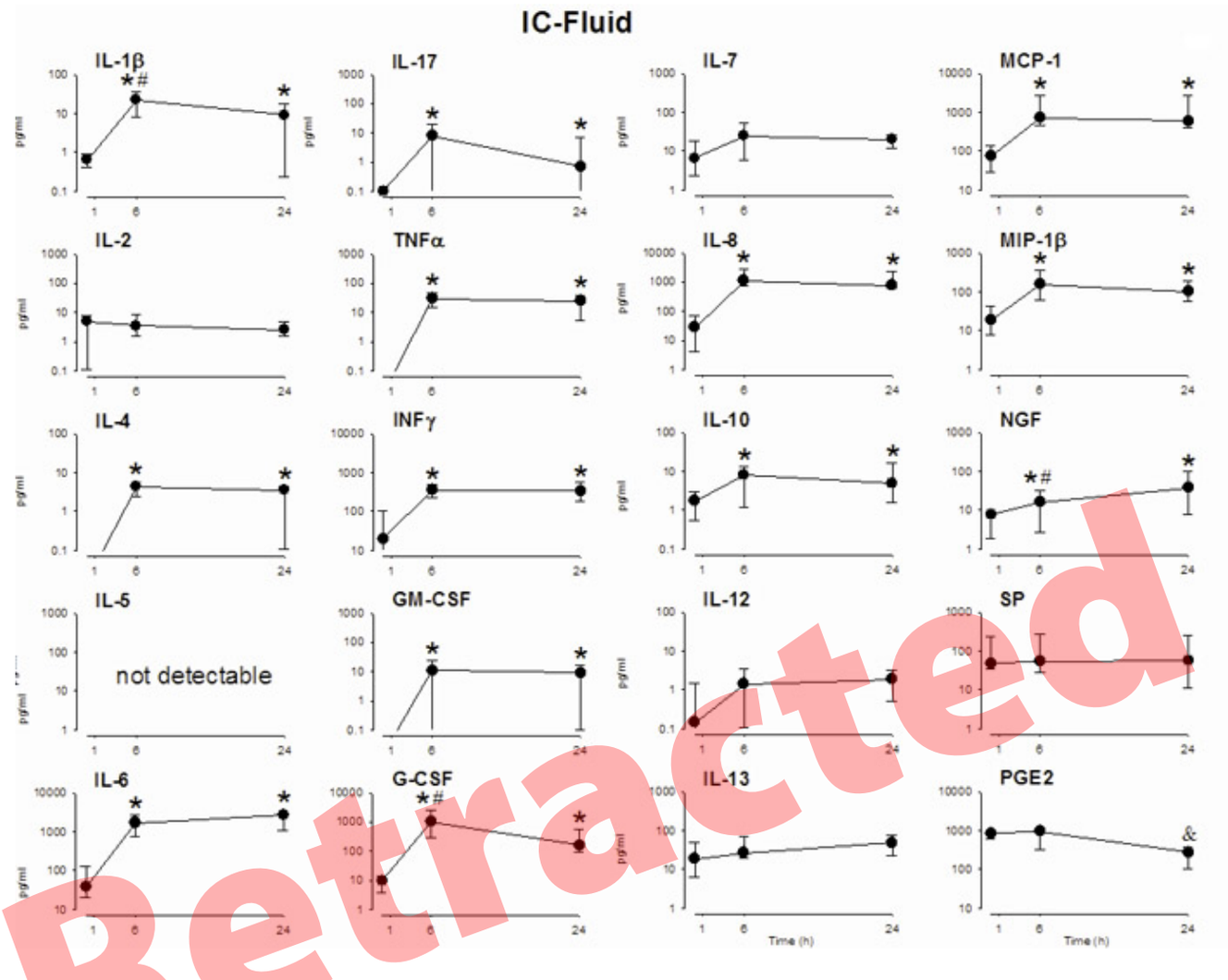
Protocol

Nociceptive and inflammatory biochemical mediator collection

1. The On-Q® PainBuster® Pain Relief System is inserted into the subcutaneous layer by the surgical team just prior to wound closure. The system continuously delivers normal saline (or local anesthetic) subcutaneously into the wound at a rate of 2 ml/h.
2. A three-way stopcock is incorporated into this system to allow aspiration of wound exudate at specified time points.
3. At time points specified by the protocol (e.g., 1, 6, 24, and 48 hours after cesarean delivery), 1 ml of wound exudate is withdrawn into a polyethylene cup containing 30 µl of proteinase inhibitor.
4. At the same time intervals 10 ml of blood is collected into a green top blood collection tube containing lithium heparin. 300 µl of proteinase inhibitor is then added to the blood samples.
5. Within 1 hour of collection, samples are put on ice and centrifuged at 3000 rpm for 10 min.
6. The serum and wound supranate is removed and place in a standard microcentrifuge tube and stored at -20°C.

Assay analysis

1. Once all samples are collected, they are thawed and analyzed at the same time.
2. Cytokines are then measured using a17-multiplex bead array immunoassay plate. Multiplex immuno-assay technology allows assaying up to 100 analytes in body-fluid samples as small in volume as 50 µl and produce results comparable with those obtained with ELISA. This plate is capable of measuring interleukin 1ß (IL-1ß), interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 7 (IL-7), interleukin 8 (IL-8), interleukin (IL-10), interleukin 12 (IL-12), interleukin 13 (IL-13), interleukin 17 (IL-17), tumor necrosis factor □ (TNF□), interferon □ (INF□), granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), monocyte chemoattractant protein 1 (MCP-1) and macrophage inflammatory protein 1 (MIP-1□).
3. Nerve growth factor is measured with NGF antibody DY256 by adding it to the 17-plex plate with aid of the Bio-Plex amine coupling kit.
4. Each measurement is made in duplicates and according to the manufacturer's specification. Standard curves for each analyte are generated by using the reference analytes supplied by the manufacturers at concentrations of 0.20, 0.78, 3.13, 12.5, 50, 200, 800, 3200 pg/ml plus a zero standard (normal saline only). Standard curves are included in each run and sample concentrations were calculated with Bio-Plex Manager software.
5. Prostaglandin E2 and substance P are measured using a highly sensitivity ELISA Kits Each assay is performed in duplicate according to the manufacturer's specification.


IC-Fluid
IL-1β
IL-17
IL-7
MCP-1
IL-2
TNFα
IL-8
MIP-1β
IL-4
INFγ
IL-10
NGF
IL-5
not detectable
GM-CSF
IL-12
SP
IL-6
G-CSF
IL-13
PGE2
pg/ml
Time (h)

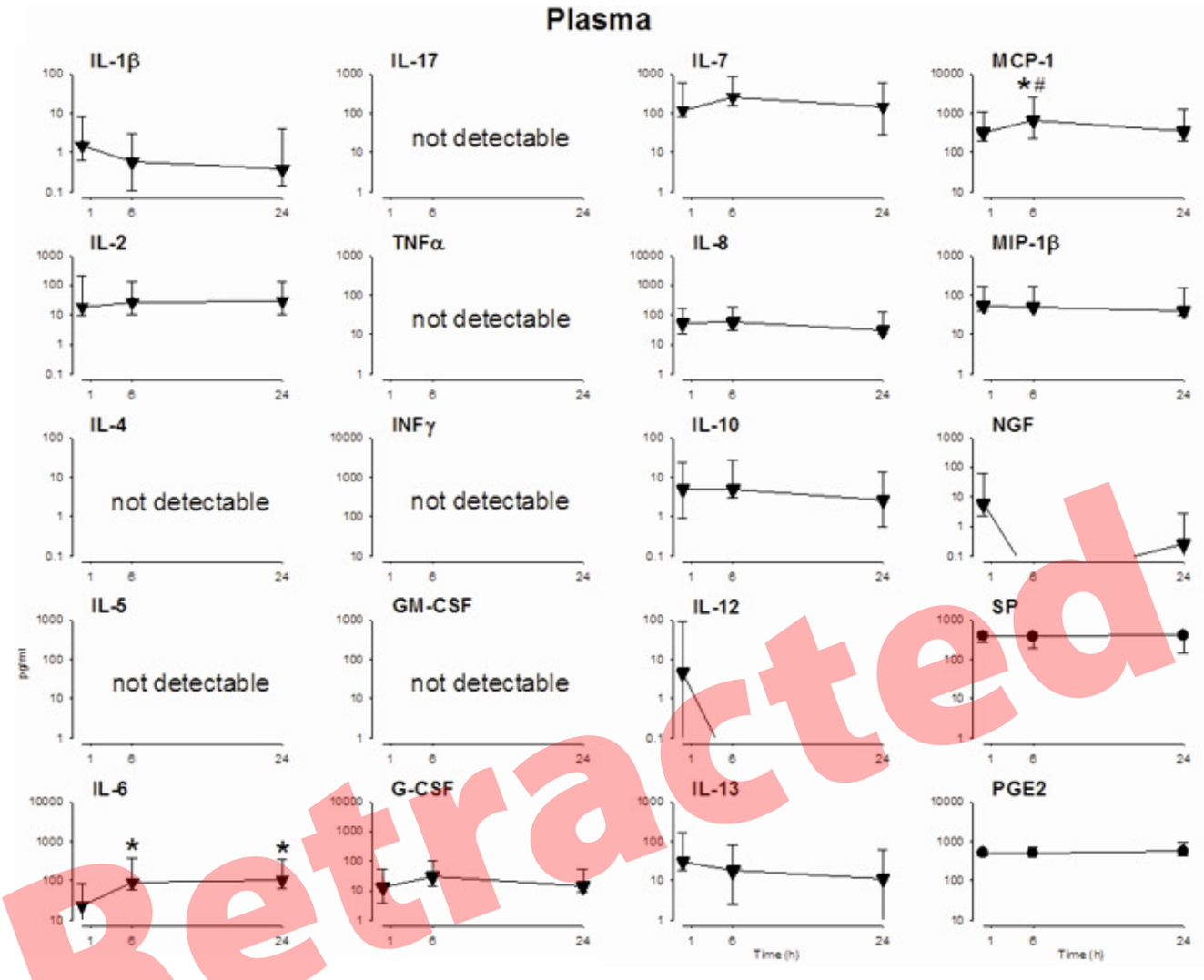


Figure 1: Exudate and serum levels of various pro- and anti-inflammatory cytokines (interleukin 1ß (IL-1ß), interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 7 (IL-7), interleukin 8 (IL-8), interleukin (IL-10), interleukin 12 (IL-12), interleukin 13 (IL-13), interleukin 17 (IL-17), tumor necrosis factor □ (TNF-□), interferon □ (INF□), granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), monocyte chemoattractant protein 1 (MCP-1) and macrophage inflammatory protein 1 (MIP-1ß), nerve growth factor (NGF), prostaglandin E2 (PG-E2) and substance P (SP) levels (pg/mL) measured at baseline, 6 and 24 hours post-cesarean delivery.

Discussion

The On-Q® PainBuster® Pain Relief System should be inserted across the entire incision in subcutaneous layer just prior to wound closure. This facilitates aspiration via the three-way stopcock at the specified time intervals. The On-Q® system continuously delivers normal saline subcutaneously into the wound at a rate of 2 ml/h. This prevents the catheter clotting and improves the reliability of the system to produce exudate samples. If aspiration of exudate is difficult (approximately 5% of cases), consider changing the subject's position (e.g., sitting the patient up or lying them flat), pushing gently above the wound, using a 0.5-1ml normal saline flush or withdrawing the catheter 1-2 cm.

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