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articoli originali

Production of cytokines at the operation site

G. DI VITA, R. PATTI, G. VETRI, B. MACCHIARELLA, P. D'AGOSTINO[1], G. CARUSO[2], V. FERLAZZO[2], F. ARCOLEO[2], S. SAMMARTANO, M. ANGILERI, E. CILLARI[2]

SUMMARY: Production of cytokines at the operation site.

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Background and aim*: Cytokines are part of a family of molecules involved in the initiation, control and termination of the events that occurs in wound healing process. Aim of this study was to evaluate the production of some cytokines [interleukin (IL)-6, IL-10, IL-1a, IL-1ra, interferon (IFN)-g] in the drainage wound fluid from patients undergoing incisional hernia repair.*

Methods: *Ten female patients with abdominal midline incisional hernia undergoing to surgical repair were included in this study. In all cases a closed suction drain was placed in the wound below the fascia and it was removed on the 4 th postoperative day. Wound fluid was collected on the 1st, 2nd, 3rd and 4th day and its amount in each time was recorded. The production of IL-6, IL-10, IL-1a, IL-1ra and IFN-g were evaluated as quantity produced in 24 hour.*

Results: *In all patients the amount of drain fluid from surgical wound was highest on the 1st day after surgery, afterwards there is a significant reduction. The production of all cytokines evaluated was highest on the 1st day decreasing on the 2nd day except for IL-1a that not show any modification. The produciton of IL-1ra, IL-6, IL-1a and IL-10 was significantly reduced on the 3rd and 4th postoperative day in comparison with the respectively values recorded on the 1st day, whereas IFN-g levels were similar.*

Conclusions: *The dosage of cytokines in the drain fluid led us to better evaluated the events that follow surgical wound and their analysis offers further information in the role of cytokines in healing process, with the goal to get supportive treatments to promote the best evolution.*

RIASSUNTO: **Valutazione della produzione di citochine nella sede dell'intervento chirurgico.**

G. DI VITA, R. PATTI, G. VETRI, B. MACCHIARELLA, P. D'AGOSTINO, G. CARUSO, V. FERLAZZO, F. ARCOLEO, S. SAMMARTANO, M. ANGILERI, E. CILLARI

Premessa e scopo: *Le citochine fanno parte di una famiglia di molecole coinvolte nel processo che regola gli eventi che portano alla guarigione delle ferite. Lo scopo del nostro studio è stato quello di valutare la produzione di alcune citochine [interleuchina (IL)-6, IL-10, IL-1a, IL-1ra ed interferone (IFN)-g], nel fluido di drenaggio di pazienti sottoposti ad intervento chirurgico per laparocele mediano.*

Metodi: *Venti pazienti di sesso femminile, affette da laparocele mediano della parete addominale, sono stati inclusi in questo studio e sottoposte ad intervento chirurgico. In tutti i casi alla fine dell'intervento è stato posizionato un drenaggio in aspirazione al di sopra della fascia, che è stato rimosso in quarta giornata. La quantità di fluido di drenaggio prodotta è stata registrata ogni giorno. La produzione di IL-6, IL-10, IL-1a, IL-1ra e di IFN-g nel fluido di drnaggio è stata valutata ogni giorno considerando la quantità prodotta in 24 ore.*

Risultati: *In prima giornata post-operatoria, la quantità di fluido di drenaggio prodotta è risultata elevata in tutte le pazienti, successivamente è stata osservata una riduzione statisticamente significativa. La produzione di tutte le citochine è risultata elevata in prima giornata post-operatoria. In seconda giornata tutte le citochine tranne l'IL-1a sono risultate ridotte (P<0,05). Rispetto ai valori osservati in prima giornata, la produzione di IL-1ra, IL-6, IL-1a e IL-10 è risultata significativamente ridotta in terza e quarta giornata. I livelli di IFN-g, invece, sono risultati simili.*

Conclusioni: *Il dosaggio delle citochine nel fluido di drenaggio consente di comprendere meglio gli eventi che si susseguono nella ferita chirurgica e la loro analisi fornisce ulteriori informazioni riguardo al ruolo delle citochine nel processo di guarigione delle ferite. La conoscenza della produzione delle citochine è importante perché possono essere utilizzate come trattamenti di supporto per favorire una migliore evoluzione delle ferite.*

KEY WORDS: Cytokines - Incisional hernia.
Citochine - Laparocele.

Introduction

Acute wounds normally heal by a physiologic patway characterized by distinct, but overlapping phases: hemostasis, inflammation, proliferation, angiogenesis, and resolution. The inflammatory phase of wound healing is characterized by the presence of neutrophils,

Università degli Studi di Palermo
Dipartimento di Discipline Chirurgiche ed Oncologiche
Divisione di Chirurgia Generale
(Direttore: Prof. G. Di Vita)
[1] Azienda Ospedaliera Universitaria Policlinico "Paolo Giaccone"
Servizio di Immuno-Ematologia
(Direttore: Prof. F. Dieli)
[2] Azienda Ospedaliera "V. Cervello"
UOC di Patologia Clinica
(Direttore: Prof. E. Cillari)

TABLE 1 - PATIENTS CHARACTERISTICS.

Age (years)°	46±16
Gender (M/F)	0/10
Anaesthesiologic grading (ASA)	
I	6
II	4
Body mass index (kg/m^2)°	26±9
Duration of operation (min)°	63±18
Diameter of hernial orifice (cm)°	4.8±2.2

°*data are expressed as mean ± standard deviation (SD)*

macrophages and lymphocytes. The inflammatory cells the seve to release proinflammatory cytokines and growth factor, ingest foreing materials, increase vascular permeability and promote fibroblast activity (1, 2).

Cytokines are part of a family of molecules that have autocrine, paracrine, and endocrine effects (3). They are involved in the initiation, control, and termination of the cellular events that occurs at each stage of wound healing, primarly by their chemotactic and mitogen properties, attracting and for stimulating cellular proliferation (4). The cytokines can be identified and quantified in the sera, in the wound drainage fluid and in the wound tissue.

Different are experimental and clinical studies that evaluate the kinetics and dynamics of cytokines involved in the chronic wound healing of the skin, while the clinical and experimental studies regarding non cutaneous surgical wound healing are few and conflicting. Baker et al. (5) reported that, in intraperitoneal fluid after elective surgery for colorectal cancer, the pro-inlfammatory cytokines IL-6, TNF-a, and IL-1a showed high levels on day 1 corresponding to the initial inflammatory response. IL-6 levels then decrease down to day 8, TNF-a levels remained static, and IL-1a levels showed a second peak on post-operative day 6 (5). In contrast Bertram et al. (6), in their study performed in 25 patients operated always for neoplastic colorectal disease, showed a significant rise in peritoneal fluid TNF-a with maximum on 7th day during the study period, while peritoneal fluid levels of IL-6 remained constant without significant change in the time. Holzheimer and Steinmetz (7) in patients undergoing an elective reduction mammoplasty have detected moderately increased levels of cytokines only in the sera; whereas in wound fluid the cytokines levels were several fold higher. IL-6 in the wound fluid peaked at 7 hours after operation. IL-8 after 4 hours and sTNFR at the second postoperative day (7). Chow et al. (8) found that, after radical modified mastectomy for cancer, only IL-6 levels in drain fluid were elevated during the initial phase, but in the later phase the IL-6 levels dropped with a corresponding rise in TNF-a levels.

The analysis of cytokines appears to be relevant to better understand the evolution of wound healing and their dysregulation has been implicated in the pathogenesis of fibrosis and delayed wound healing (9).

In the present study we have evaluated the production of some cytokines (IL-6, IL-10, IL-1a, IL-1ra, IFN-g) in the drainage wound fluid after surgical repair of incisional abdominal midline hernia to better recognize the complexity of healing process and the possible application of some of these factors in modulating positive healing.

Patients and methods

Study population

Ten female patients with abdominal midline incisional hernia admitted for surgical repair were included in this study. The incisional hernias had developed at the site of a previous vertical subumbilical incision after hysterectomy for leiomyoma. Patients with metabolic, endocrine, hepatic or renal disease or those receiving medication know to interfere with wound healing were excluded from the study. Size of hernial defect, age, gender, anaesthesiologic grading, duration of operation and body mass index are given in table I.

The patients received the same standard anaesthetic procedure using thiopental sodium for induction, vencuronium for neuromuscolar blockade, isoflurane and fentanyl citrate for analgesia. All operations were performed removing the previous surgical skin scar. The hernial sac, isolated from related structures, was never resected. In every patients a straight suture of fascia and muscles was performed using Dexon sutures (Davis-Geck, Wayne, NJ, USA). In all cases, a closed suction drain (Redax, Mirandola, Italy) was placed in the wound below the fascia.

The patients were given a perioperative antibiotic (1 g ceftazidime) and low-molecular weight heparin (enoxaparine sodium, 4.000 IU daily during the study period) prophylaxis. The wound drain was removed on 4th postoperative day and the patients were discharged on the 5th-6th day.

Cultures of wounds and wound drainage fluid were to be obtained only when there was a suspicion of infection (notably wound edema, erythema or serosanguinos discharge) and during this study was not required in any patient.

All patients, prior to their inclusion in the study, gave written informed consent and the local ethics committee approved the study.

Wound fluid samples and assays

Surgical wound fluid was collected in a closed sterile collection bag which was replaced daily with a new sterile bag under aseptic conditions. Wound fluid was collected at 1st day after the operation and thereafter 2nd, 3rd and 4th days postoperatively. The amounts of wound fluid per each patient in each time was thorougly recorded. Wound fluid samples were centrifuged at 2,000 g for 10 minutes and stored at - 70 °C until assay. The cytokines and growth factors were evaluated as quantity produced in 24 hours. Value were obtained multiplicand their concentration per the volume collected in 24 hours.

Enzyme-linked immunosorbent assay (ELISA) commercial kits were used to determine the production of IFN-g (CLB, Amsterdam The Netherlands) and IL-6, IL-10, IL-1a, IL-1ra (Euro Clone Ltd, UK). The Netherlands wound fluid samples were diluted where necessary and the ELISA performed following the assay protocol.

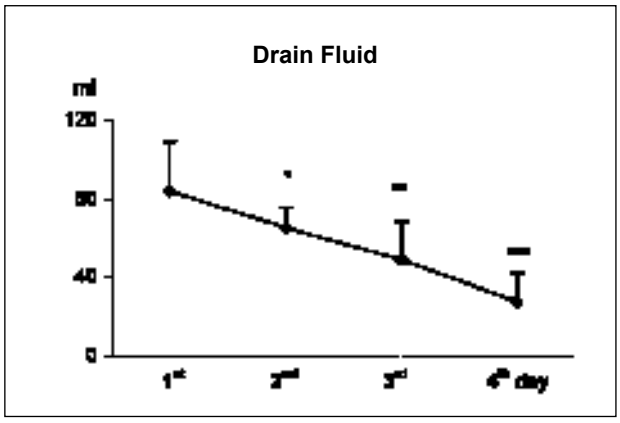

Fig. 1 - The amount of drainage fluid of wound healing collected in 24 hours at different days after surgery. Values are expressed as mean ± standard deviation (SD). Statistical significance was analysed by ANOVA. P<0.05 was considered statistically significant. Significant differences vs 1st postoperative day: * P<0.05; ** P<0.01; *** P<0.001.

The optical density for each well was determined using a microplate reader set at 450 nm with correction wavelength set at 540 nm.

Statistical analysis

All samples were assayed in duplicate and the mean value was used for calculation of results. All data are presented as mean ± standard deviation (SD). Statistical significance was analysed by ANOVA and P<0.05 was considered statistically significant.

Results

During the study period, all patients showed an uncomplicated intra-operative course and there were no postoperative septic or local complications of the surgical wound, notably hematoma, infection, abscess formation or dehiscence. At distance of six months from surgical procedure, clinical and eco-tomographics studies not shown signs of recurrences.

The amount of drain fluid from surgical scar was 83.75±25.03 ml on the 1st day postoperatively, whereas on the 2nd 3rd and 4th day there was a significant reduction (respectively P<0.05, P<0.01, P<0.001) (Fig. 1).

The production of all cytokines resulted higher on the 1st postoperative day (Fig. 2). The amount of IL-1ra, IL-6, IL-10 and IFN-g produced on the 2nd postoperative day in comparison with that detected on the 1st day resulted reduced (P<0.05), whereas it was not observed any modification in the amount of IL-1a (Fig. 2). The production of IL-1ra, IL-6, IL-1a and IL-10 was significantly reduced on the 3rd and 4th postoperative day in comparison with the respectively values recorded on the 1st day, whereas IFN-g levels were similar (Fig. 2).

Discussion

We previously reported that abdominal midline incisional hernia repair is a suitable model for studying the acute inflammatory response after surgery (10). The surgical dissection results in significant tissue damage, with very low rate of bacterial contamination. We showed that there was significant elevation of leukocyte and neutrophil counts after incisional hernia repair, together with high serum levels of IL-6, C-reactive protein and fibrinogen were elevated (10). The changes were prolonged and represented the total bodily responses to surgical trauma in addition to local inflammatory responses at the operation site (11).

To gain insight in the cytokine and growth factor dynamics in the wound evolution after incisional hernia repair, in the present study we have concentrated our attention on the changes of these mediators in the wound fluid. The post-operative events at the operation site are rather complex. While the immediate and early local responses are part of the inflammatory and perhaps acute phase reactions to acute surgical trauma, the subsequent events were related to wound healing.

Our results indicate that inflammatory cytokines (IL-1a, IL-6, IFN-g) are in high concentration in the fluid drainage in first 24 hours, afterwards they decline. In agreement, there is a precocious and elevated production of anti-inflammatory cytokines (IL-10, IL-1ra) to counterbalance acute response. The early production (24-48 hours) of IL-1a together with the decrease of it within the third day is in line with the essential role of this cytokine in the healing process, since it increases collagen synthesis as well as stimulates keratinocyte and fibrolast growth (12). In fact, the persistence of high levels of IL-1a after the first week are deleterious and pathogenic (13,14). The secretion of IL-1ra parallels the secretion of IL-1a, functioning as a competitive inhibitor of IL-1 receptor binding *in vitro* and *in vivo*, antagonizing the activities of both molecular forms of IL-1 without having agonist effects itself (15). The detection in the wound fluid of high levels of IL-6 at 1st day, with a progressive reduction in the following days, is the expression of both local and systemic effect of wound healing (16). Also the secretion of IFN-g appears to follow the normal evolution of the wound, inducing tissue remodelling by the collagenase expression (17, 18).

Some of our results are in apparent disagreement with those reported by others (5-8). Although, it must to be note that we have evaluated the amount of cytokines produced in 24 hours before the sampling, while in the others studies it was evaluated only cytokines concentration without considering the amount of wound drain fluid that, in absence of complications – as we observed – decreased significantly in the postoperative period (P<0.001 on 4th day).

The dosage of cytokines in the drain fluid led us to better evaluated the events that follow surgical wound (19). The presence of a drain near the suture can itself modify the profiles of cytokines and wound healing.

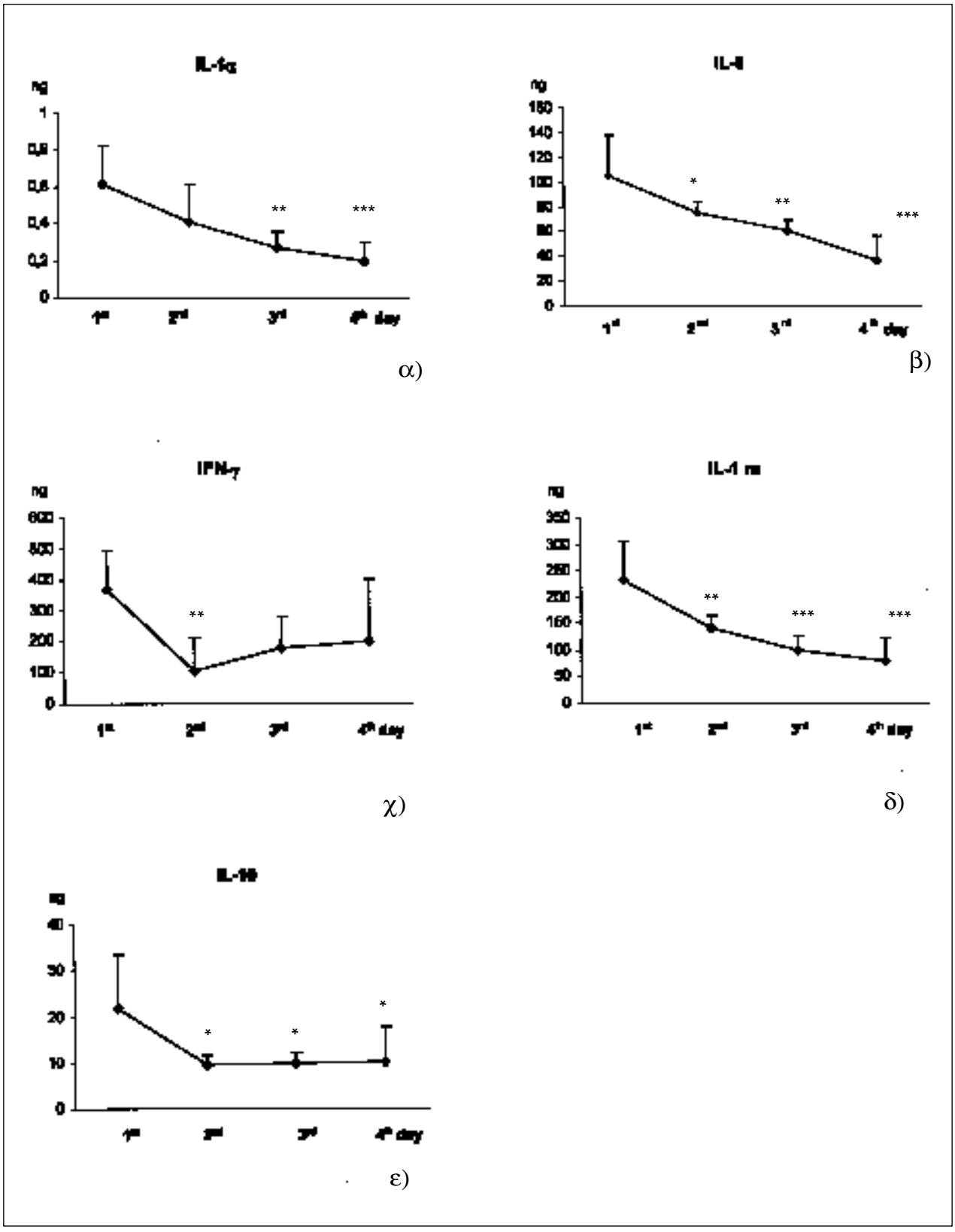

Fig. 2 - The amount of IL-1a (a), IL-6 (b), IFN-g (c), IL-1ra (d), IL-10 (e), expressed in ng, produced in 24 hours at different days after surgery. Values are expressed as mean ± standard deviation (SD). Statistical significance was analysed by ANOVA. P<0.05 was considered statistically significant. Significant differences vs 1st postoperativa day: * P<0.05; ** P<0.01; *** P<0.001.

However, the levels of cytokines secreted near the surgical wound can be determined only using a drainage. Drain fluid, probably is still the best clinical surrogate marker of healing (4).

Conclusions

The knolewdge of dynamics of cytokines secretion profiles in the site of surgical operation is important because the cytokines make a crucial role in the physiopathology of wound healing and they are a popular target for modifying the repair response (20). In fact, the addition or the neutralization of a specific proinflammatory cytokines may be effective when used at one time point in the healing process, continued active antagonism or supplementation may not produce similar results (20). These data explain the apparent paradoxical nature of many results with cytokines inhibition or supplementation (3). Fetal mammalian wounds have almost undetectable levels of IL-6 and IL-8, and IL-6 administration resulted in strong scar formation (21, 22). IL-1 is undergoing to clinical evaluation for pressure ulcers (23) whereas IFN-g, because decreasing collagen production, could be used for the treatment of hypertrophic scars (24).

In conclusion in all our patients the analysis of cytokines offers further informations in the role of cytokines process, with the goal to get supportive treatments to promote the best evolution. The complexity of the wound healing will require further understanding before the therapeutic potential of cytokines is fully recognized (20).

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