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The Postoperative Stress Response and Its Reflection in Cytokine Network and Leptin Plasma levels

I. CHACHKHIANI[1], R. GÜRLICH[1], P. MARUNA[2], R. FRAŠKO[1], J. LINDNER[3]

[1]First Surgical Department, [2]Department of Pathological Physiology and [3]Second Surgical Department of Cardiovascular Surgery, First Faculty of Medicine, Charles University, Prague, Czech Republic

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Summary

The objective of the present report was to clarify the postoperative stress response of some inflammatory markers, namely of proinflammatory cytokines and leptin levels during uncomplicated postoperative periods. The results were compared with the dynamics of these parameters during intraabdominal sepsis. We followed 20 patients after a planned resection of colorectal cancer in stage Ib-IV with uncomplicated healing and 13 obese men after laparoscopic non-adjustable gastric banding. These were compared to 12 patients with proven postoperative sepsis. The control group consisted of 18 healthy men. The observed parameters included serum levels of cytokines, tumor necrosis factor-α (TNFα), interleukin-1β (IL-1β), interleukin-1 receptor antagonist (IL-1ra), IL-6, IL-8, soluble receptor of interleukin-2 (sIL-2R) and leptin. It was found that during the first 24 h after resection there was a significant increase in the serum concentration of IL-6 up to 1125±240 ng/l, which declined within the next 48-72 h. Serum concentration of TNFα was highest 18-24 h after resection (205±22 ng/l) and after banding (184±77 ng/l). IL-1β had a stable serum concentration without significant elevation. Serum concentration of IL-8 after resection rose to 520±200 ng/l after 36-48 h. Maximal cytokine levels after gastric banding were quantitatively lower (IL-6 414±240 ng/l, TNFα 184±77 ng/l) than after resection. We found significant elevation of plasma leptin concentration (32±10 ng/ml) 24 h after banding compared with preoperative values (18±5 ng/ml, $p<0.05$). Leptin levels 48 and 72 h after banding rapidly returned to the level before operation. During abdominal surgery leptin shows to be an acute phase reactant. Proinflammatory cytokines can be main regulatory factors of leptin during this period. Significant correlation between leptin and TNFα (similarly demonstrated by other authors in models of bacterial inflammation) indicates that TNFα can be the crucial regulator of leptin generation in the early postoperative period. On the basis of our results we recommend to observe IL-6 and IL-8 at 24-72 h after the surgery in patients with a high risk of early postoperative septic complications.

Key words
Cytokines • Leptin • Systemic inflammatory reaction • Surgical stress

Introduction

Each surgical operation is followed by various degree of tissue injury and tissue ischemia. The injury starts with the interference of the integrity of organism and causes local and systemic reactions, essential for

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

defense, adaptive and reparative processes.

It is not just the severity of the environmental insult that determines the extent of the resulting injury, but it is rather the injury which occurs within the context of the immune response to the insult. Physiologically, this response operates in a restricted environment to eradicate infected or devitalized tissue. If the inflammatory response escapes local control, it induces a generalized systemic response. Excessive or prolonged activation of macrophages and neutrophils results in inappropriate production of cytokines (Bahrami and Redl 1991, Cheadle and Mercer 1996). Cytokines are low molecular weight proteins, which are potent at low concentrations. After binding to specific receptors, cytokines initiate multiple effects, some of which include synthesis of other cytokines. Cytokine release can occur in several phases with an initial local release of interleukin-1 (IL-1) and tumor necrosis factor-α (TNFα) followed by a secondary more distant cytokine release. These proinflammatory cytokines influence practically all organs, increase the expression of vascular adhesion molecules and they elevate the *in vitro* production of eicosanoids and the platelet activating factor. They accelerate the inflammatory response by activating inflammatory and endothelial cells in vascular compartments remote from the initial sites of inflammation. The increasingly dysregulated and misdirected immune response leads to whole-body inflammation, coagulopathy and organ injury associated with systemic inflammatory response syndrome (SIRS). This causes additional release of cytokines, and the host enters a spiral of increasing organ dysfunction. Some cytokines including IL-1, IL-6, IL-8 and TNFα directly cause nonspecific injury to cells. (Ahmed *et al.* 1995, Ahmed and Christou 1996, Mayers and Johnson 1998).

Leptin is a product of fatty cells and due to endocrine effects is involved in the mechanism of the body fat content regulation and body weight maintenance. Leptin receptors are situated mostly in the pituitary, but they have also been found in other tissues. Through its receptors leptin induces a number of metabolic and endocrine effects. Leptin levels are increased almost in all obese patients, in which they correlate well with body mass index (BMI) and body fat content. It is assumed, although experimentally not clearly corroborated, that the increase of leptin during the systemic inflammatory reaction contributes to anorexia (Jiskra *et al.* 2000) and cachexia and it accompanies acute and chronic inflammatory disorders (Plata-Salaman 1998, Pralong *et al.* 1998). Similarly, leptin with characteristics of an endogenous pyrogen, can potentiate a pyrogen effect of IL-1 through its receptors in the hypothalamus. Finally, the stimulating hematopoetic effect of leptin (Haluzík *et al.* 2000a,b) and its stimulating influence in angiogenesis, detectable *in vitro*, can modify the course of the acute and reparative phase of inflammatory reaction (Sierra-Honigmann *et al.* 1998). Leptin by structural, evolutional and functional characteristics is close to IL-6 and to IL-6-related cytokines such as IL-13, onkostatin M, and the granulocyte colony-stimulating factor.

The systemic release of inflammatory cytokines occurs several hours earlier than the release of other markers of systemic inflammation such as acute phase proteins (APP) and leukocytosis, suggesting their potential importance as diagnostic parameters in SIRS and post-surgery sepsis.

In this study we evaluate the dynamics of leptin and cytokines acute changes in two model situations: in the early postoperative period (in patients after laparoscopic nonadjustable gastric banding and after operation of colorectal carcinoma) without infectious complications and in patients with sepsis.

Methods

Forty-five patients who underwent surgery at the First Department of Surgery of the First Faculty of Medicine were examined: 1) patients after planned resection of colorectal cancer in stage Ib-IV with uncomplicated healing (20 men, aged 58±92 years), 2) 13 obese men, aged 34-51 y, (stage of obesity by BMI) indicated for laparoscopic nonadjustable gastric banding**,** 3) patients with proved postoperative intra-abdominal sepsis (12 men, 59±8.9 years), and 4) a control group consisted of 18 men – all healthy persons, aged 35-50 years. In our study, we chose laparoscopic nonadjustable gastric banding and colorectal cancer resection as models of an uncomplicated course, because these groups differ from each other both in the intensity of noninfectious inflammatory stimuli and initial leptin levels. In our study we investigated patients of the same gender (men) because of different physiological levels of leptin in men and women.

Postoperative serum levels of IL-1β, IL-6, IL-8, interleukin-1 receptor antagonist (IL-1ra), soluble receptor of interleukin-2 (sIL-2R) and TNFα were measured by ELISA kits (Imunotech). Plasma concentrations of leptin

were determined by ELISA (Bio Vendor). The sensitivity of the method is 0.5 ng/ml.

The levels of cytokines and leptin were measured before the operation and at 12, 24, 36, 48 and 72 h after the start of the operation (groups 1 and 2), and compared with levels found in sepsis (group 3, measured only once, on the second day after the onset of sepsis).

The data are given as means ± S.D. Statistical analysis was performed by Student's test; a $p<0.05$ value was considered to be significant. Correlation between individual values for cytokines and leptin was analyzed by linear regression. The multiple comparison test was performed for evaluating the differences among tested groups.

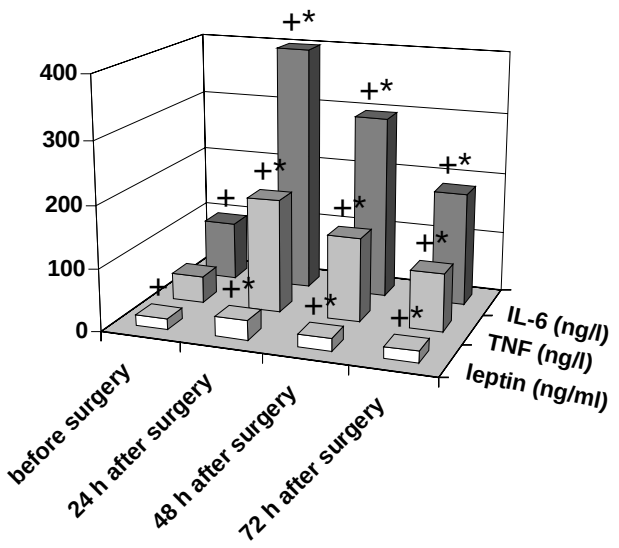

Fig. 1. The dynamics of plasma leptin, TNF-α and IL-6 after gastric banding (mean levels). + = statistical significance of difference against control group ($p<0.05$); * = statistical significance of difference against initial preoperative levels ($p<0.05$). Data are shown as mean ± S.D.

Results

During the first 24 h after resection there was a significant increase in serum concentrations of IL-6 to 1125±240 ng/l (vs. 3258±1845 ng/ml, $p<0.01$ in the septic group), which declined during the next 48 to 72 h (Table 1). Serum concentrations of TNFα was highest 18-24 h after resection (205±22 ng/l vs. 415±225 ng/l, $p<0.05$). IL-1β serum concentrations were stable without significant elevation. At 36-48 h after the surgery serum concentration of IL-8 rose to 520±200 ng/l (vs. 916±322 ng/l, $p<0.05$) (Table 1). In groups 1 and 2, the levels of IL-6 were already elevated 12 h after surgery with a maximum achieved after 24 h (Tables 1 and 2). This was followed by a mild decrease at 48 h and a more pronounced decrease at 72 h. The concentration of TNFα in the septic patients was highest and were significantly different both from the control group ($p<0.001$), and from the maximal values of groups 1 and 2 ($p<0.01$).

The dynamics of plasma leptin, TNFα and IL-6 values after the surgery are presented in Figure 1. We have demonstrated a significant elevation of plasma leptin concentrations 24 h after the operation in both group 1 and 2 compared with the preoperative status. Leptin levels 48 and 72 h after banding quickly returned to values before the operation.

Student´s multiple comparison test confirmed a significant difference between pre-banding and post-banding (+24 h) leptin, TNFα, sIL-2R, and IL-6 ($p<0.05$, $p<0.01$, $p<0.05$ and $p<0.001$, respectively).

Figure 2 shows the correlation between leptin and TNFα 24 h after laparoscopic gastric banding. The correlation significant for leptin and TNF-α 24 h after surgery ($r=0.40$, $p<0.05$), and for leptin and IL-6 24 h after surgery ($r=0.29$, $p<0.05$). There were no significant correlations between leptin and IL-1β or leptin and sIL-2R.

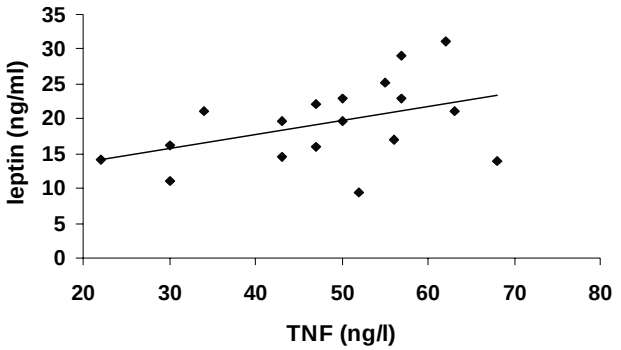

Fig. 2. The correlation between leptin and TNFα 24 h after laparoscopic gastric banding ($y = 0.19 x + 8.22$; $r=0.40$, $p<0.05$).

A significant increase of leptin 24 h after surgery was found in groups 1 and 2 compared to values before surgery ($p<0.01$ and $p<0.05$, respectively) and compared to the control group ($p<0.01$ and $p<0.001$, respectively) (Tables 1 and 2). In group 1, but not in group 2, a transient decline of leptin concentrations 12 h after surgery was found ($p<0.05$). In both groups, plasmatic concentrations of leptin measured 48 and 72 h after surgery did not differ from the levels before surgery. The elevation of leptin levels in the septic patients (group 3) was comparable with the maximal levels in group 2 during the post-operative period, or it may exceed maximal levels of leptin in group 1.

Table 1. The changes of cytokines and leptin after resection of colorectal cancer.

Cytokines	Before operation	+12 h	+24 h	+36 h	+48 h	+72 h	Postoperative sepsis	Controls
IL-1β (ng/l)	31.2±4.2	37.9±8.3^{+}*	37.6±7.8^{+}*	36.5±4.8^{+}*	35.4±2.6^{+}*	33.2±4.7^{+}*	41.2±8.2	27.2±4.6
IL-1ra (ng/l)	386±84	2266±218^{+}*	2288±314*	1066±322^{+}*	812±288^{+}*	616±242^{+}*	2517±542^{+}*	288±88
sIL-2R (ng/l)	573±188	2265±542^{+}*	3910±512^{+}*	3854±544^{+}*	2854±486^{+}*	1950±451^{+}*	4012±941^{+}*	512±166
IL-6 (ng/l)	81±12^{+}	950±140^{+}*	1125±240^{+}*	1000±180^{+}*	840±120^{+}*	200±85^{+}*	3258±984^{+}*	12±8
IL-8 (ng/l)	39.4±12.4	120±48^{+}*	430±180^{+}*	520±200^{+}*	480±195^{+}*	250±72^{+}*	916±322^{+}*	24.2±8.2
TNFα (ng/l)	63.9±8.9^{+}	150±18^{+}*	205±22+*	180±20^{+}*	120±16^{+}*	70±10^{+}*	415±225^{+}*	46±5.3
Leptin (ng/l)	4.1±1.8	3.0±1.1^{+}*	27.5±8.6^{+}*	–	7.9±3.9^{+}*	4.8±3.0^{+}*	36.8±11.2^{+}*	4.5±2.2

Data are means ± S.D. $^{+}$ significantly different from control group ($p<0.05$); * significantly different from initial preoperative levels ($p<0.05$).

Table 2. The changes of cytokines and leptin after gastric banding.

Cytokines	Before operation	+12 h	+24 h	+36 h	+48 h	+72 h
IL-1β (ng/l)	31.3±4.1	36.7±8.4^{+}*	37.4±7.5^{+}*	35.6±4.7^{+}*	34.3±2.5^{+}*	33.3±4.5^{+}*
IL-1ra (ng/l)	564±62^{+}	2166±238^{+}*	2208±284^{+}*	1084±324^{+}*	914±284^{+}*	718±247
sIL-2R (ng/l)	588±176	2365±523^{+}*	3810±543^{+}*	3754±487^{+}*	2654±376^{+}*	1650±254^{+}*
IL-6 (ng/l)	96.0±64^{+}	250±78.8^{+}*	414±240.4^{+}*	324±180	296±124^{+}*	184±74.8^{+}*
IL-8 (ng/l)	39.6±11.5^{+}	124±38+*	370±175^{+}*	460±210^{+}*	420±175^{+}*	245±74^{+}*
TNFα (ng/l)	44.0±16.4	114±58.6^{+}*	184±76.5^{+}*	150±45^{+}*	136±88.8^{+}*	94.6±31.4^{+}*
Leptin (ng/l)	18.4±5.2^{+}	16.9±4.2^{+}*	32.2±10.2^{+}*	–	21.4±14.9^{+}*	19.6±8.4^{+}*

Data are means ± S.D. $^{+}$ significantly different from control group ($p<0.05$); * significantly different from initial preoperative levels ($p<0.05$).

All monitored cytokines (with the exception of leptin) reacted to the surgical trauma with an elevation 12 h after the beginning of surgery.

We also noted significant correlations of TNFα and leptin ($r=0.49$ $p<0.05$) as well as of IL-6 and leptin ($r=0.46$, $p<0.05$) in septic patients.

Discussion

The human response to surgical stress is characterized by a series of inflammatory, hormonal and metabolic changes that altogether constitute the general stress response of the acute phase reaction. The levels of cytokines and leptin are closely related with the inflammatory reaction and the anatomical extent of the inflamed tissue involved, as well as with the activity of the immune reaction (Kain *et al.* 1999). Although both basic science and clinical studies have revealed these responses, the exact mechanisms initiating, regulating and sustaining this stress response have not yet been identified. Recently, the interactions between immune and neuroendocrine systems have been investigated. These complex interactions between proinflammatory cytokines and the hypothalamo-pituito-adrenal (HPA) axis (partial synergism, partial antagonism) results in leptin synthesis and elevation of leptin plasma levels.

The initiation of systemic inflammatory response syndrome and its course and prognosis depend on the complex interaction of proinflammatory and antiinflammatory cytokines, and the co-operation of many other soluble and membrane bound mediators in balance with activation of the HPA axis.

The acute phase of the response is triggered by inflammatory mediators – cytokines with pivotal role of

TNFα and IL-1 (van der Poll and Lowry 1995), and with a central control role of IL-6 (e.g. in the induction of APP synthesis in liver). IL-6 is secreted in early stages of the acute phase response (18-24 h after the stimulus). The monitoring of IL-6, TNFα or IL-8 has the highest diagnostic value to 72 h after the operation (Heesen *et al.* 1996). It is known that the persistence of TNFα (Sheeran and Hall 1996, Tang *et al.* 1996) and IL-6 in the serum rather than peak levels of cytokines reveals the onset of sepsis and predicts a poor outcome in septic patients (Pinsky *et al.* 1993, Ertel and Kremer 1995)

According to contemporary knowledge leptin is involved in the network of inflammatory mediators. During SIRS, its plasma concentration increases by the action of inflammatory mediators (Kain *et al.* 1999). The mechanism of this inflammatory induction is not known. In *in vitro* experiments the activation cascade includes IL-1β (Faggioni *et al.* 1998), but not IL-6. IL-1β paradoxically inhibits *in vitro* the synthesis of leptin, while *in vivo* its synthesis is activated.

Our study confirms that the dynamics of leptin in the postoperative period is characteristic for the reactants of the acute phase response. According to our previous results, the degree of leptin increase compared with its initial levels is related to the type of surgery (Kaška and Živný 2003). The basic levels of leptin are higher before laparoscopic banding than before resection of colorectal cancer. Maximal levels in the postoperative period can reach the levels of septic patients. The concentration of leptin fails to differentiate the onset of sepsis from a non-complicated course. HPA axis is activated not only by the direct effect of cytokines (especially IL-1β) on the hypothalamus, but also by afferent nerve stimulation from the periphery. This can play a critical role especially during the surgical intervention. Our previous data (Maruna *et al.* 2001) showed a dissociation in the postoperative dynamics of leptin and BMI and a significant correlation of plasma leptin levels with those of the main proinflammatory cytokines. During non-infectious stress response (as abdominal surgery) leptin shows itself as an acute phase reactant. Significant correlation between leptin and TNFα (Yamaguchi *et al.* 1998) indicates that TNFα can be crucial regulator of leptin generation in the early postoperative period. It is possible that proinflammatory cytokines induce OB gene transcription *in vivo* through secondary mediators such as the transforming growth factor-β (Granowitz 1997).

Leptin, under physiological conditions, is an adipostatic hormone preventing fat accumulation *via* a negative feedback. But this negative feedback mechanism is altered in obese patients for various reasons (receptor or post-receptor resistance etc.). On the other hand, leptin seems to be a stress-related factor, structurally, functionally and perhaps evolutionary related to proinflammatory and hemopoietic cytokines. Proinflammatory cytokines such as TNF-α or IL-1β can be the main regulatory factors of leptin during this period. Leptin might be an important link in the inflammatory network, necessary for an adequate course of the acute phase reaction after the surgery. Induction of leptin during the surgical stress response may contribute to wound healing, postoperative infection risk, anorexia, and activation of hemopoiesis, but this role has not been confirmed in clinical situations.

Cytokines play a significant role not only in regulating pathogenic mechanisms during the onset of SIRS and the multiple organ dysfunction syndrome, but can directly lead to tissue damage. The inhibitory effect of proinflammatory cytokines, such as TNFα, IL-1β and IL-8 activate neutrophils and the vascular endothelium with increased up-regulation of adhesion molecules, setting at a train of pathogenic inflammatory reactions in the host. Increased concentrations of inflammatory cytokines observed in the initial phase of postoperative complications are of great significance in potentially forecasting the fatal outcome of the disease and can be used for the early diagnosis of systemic complications. The increased leptin concentrations in the postoperative period in septic patients reflects a different regulation of the leptin synthesis in the rest period and during the systemic inflammatory reaction. During a non-infectious stress response (as abdominal surgery), leptin itself acts as an acute phase reactant. Significant correlation between leptin and TNFα indicates that TNFα can be a crucial regulator of leptin generation in the early postoperative period.

Although this field is promising for the development of new techniques for improving the results outcomes following surgery, given methods are not sufficient due to their low specificity. Opposite to other inflammatory markers, procalcitonin, the protein consisting of 116 amino acids, is very sensitive to bacterial inflammation. In some cases, its detection is more useful than inflammatory cytokines and acute phase proteins. We assume that procalcitonin will be used in surgical practice as an early indicator of developing systemic bacterial infection (in multiple injuries, after

surgery) and for subsequent evaluation of the effectiveness of treatment of septic conditions.

Acknowledgements

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Reprint requests

R. Gurlich, The Clinic of Transplantation Surgery, IKEM 1958, 14021 Prague 4, Czech Republic, e-mail: robert.gurlich@ikem.cz