THE ROLE AND PLASMA LEVEL CHANGE OF SOMATOSTATIN IN SURGERY AND SYSTEMIC INFLAMMATORY RESPONSE SYNDROME: CLINICAL AND ANIMAL EXPERIMENTAL MODEL

Ph.D. Thesis

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1. Introduction

Systemic Inflammatory Response Syndrome (SIRS), sepsis, septic shock and multi organ failure (MOF) are the leading cause of death in the group of critically ill patients at the intensive care units. The mortality of sepsis has been reduced significantly due to the assessment and the introduction of the results of human and animal experimental models into the everyday clinical practice in the last few years *(Surviving Sepsis Campaign: International guidelines for management of severe sepsis and septic shock 2008-2012).* The pathogenesis of multi organ failure due to septic shock is not known entirely even in spite of the wide spectrum of clinical and experimental results. This is a very complex and multi-functional pathophysiological process, which has been in the focus of clinical and theoretical research. Microcirculatory failure, tissue and cell level of hypoperfusion, hypoxia and acidosis caused mitochondrial dysfunction, depletion of adenosine triphosphate (ATP) effect the whole human body and make the disintegration of it.

In clinical practice, to follow the progression of septic state and the efficacy of the therapy the plasma level of CRP and PCT is measured. There are other biomarkers (cytokines and adhesion molecules) available to be measured and to follow the pathophysiology and progression of sepsis. Interleukin 6 (IL-6), Interleukin 8 (IL-8), monocyte chemotactic protein 1 (MCP-1), the soluble CD40 ligand (sCD40L), the tissue plasminogen activator (tPA), the soluble P-selectin (sP-selectin) and the vascular cell adhesion molecule (VCAM-1) are also important biomarkers in sepsis. The plasma levels of these molecules have a very tight correlation to the progression of SIRS and sepsis.

Somatostatin is either a 14 or 28 amino acid cyclic peptide. Somatostatin, in addition to the capsaicin sensitive nerves, can be found in the peripheral nervous system, in the neuroendocrine cells of the gastrointestinal system and in inflammatory cells as well. The activated synovial and immune cells are also producing somatostatin in the joints. There is a strong neuro immune modulator function of somatostatin in the central nervous system. It is also stored and secreted by the peptiderg sensitive neurones. There is experimental evidence available that exogen somatostatin can reduce pain sensation in animal experimental model and in different pain syndromes. There are several experimental results

available that somatostatin released from capsaicin sensitive nerve endings in the blood is able to reduce systemic inflammation and has got antinociceptive effects.

Five different types of somatostatin receptors (sst_{1-5}) are available according to literature. Previous experimental results show that the analgesic and anti-inflammatory effect of somatostatin belong to sst_1 and sst_4 receptors. The stable and selective sst_4 agonists are a new therapeutic option and area in the anti-inflammatory and pain therapy processes.

Neuropeptides are released by different stimulus from the sensory nerve terminals, e.g. pro-inflammatory, anti-inflammatory and nociceptive, anti-nociceptive effects. The stimulation of the peripheral capsaicin sensitive nerve terminals results in the release of pro-inflammatory neural mediators, which make further local efferent effect in the innervated tissue. This local efferent reaction is called neurogenic inflammation. The lung and joints are particularly innervated by nociceptive C fibres without myelinated capsule and with A delta fibres. There is SOM released by the mediators from the same vesicles.

It has been proved that SOM not only takes part in the local inflammatory processes but it has got distant effect as well by regulating anti-inflammatory and anti-nociceptive reactions. The elevation of plasma SOM level is not achieved after the desensitization of the capsaicin sensitive sensory nerve terminals. By using exogen SOM it has reduced the seriousness of sepsis and improved survival. There is evidence that the plasma level of SOM is increasing significantly after laparoscopic cholecystectomy and umbilical hernia operation. Since human joints and lung also have peptidergic nociceptive fibres, human joint operation (hip and knee replacement) and chest operation (thoracotomies: TX and video assisted thoracoscopic surgery: VATS) were studied to investigate how the plasma level of SOM changed during the time of surgery under general anaesthesia. Research results indicate the protective role of SOM, therefore our investigation was extended to include septic patients, as well. The change of plasma level of SOM was also measured. The protective role of SOM and its effect on survival was also investigated in coecal ligation and puncture (CLP) experimental animal model.

2. Aim of the study

1. The measurement of plasma somatostatin concentration in pain and inflammatory processes related to surgery (knee and hip replacements and thoracotomies) and in septic patients treated at intensive care units.
2. The follow up of septic patients at intensive care unit, the measurement of plasma concentration of somatostatin on a daily basis in systemic inflammatory processes during sepsis. Similarly, to measure the change in plasma concentration of different types of adhesion molecules (CRP, PCT, IL-6, IL-8,.sCD40, MCP-1, tPA, sVCAM-1, sP-selectin) to prove septic state.
3. According to clinical results the release, origin, function of somatostatin and its effect on mortality in an experimental rat model (coecal ligation and puncture: CLP model) was examined.

3. Methods and Results

3.1. Clinical experiments

3.1.1. Materials and methods

Altogether 48 patients (20 males and 28 females) with a mean age of 59.9 years (males: 61.7, females: 58.1) were enrolled in our study which took 4 weeks. Written informed consents were obtained prior to participation in all cases. The studies were approved by the Ethics Committee of the University of Pécs.

Concerning thoracic surgery, thoracotomies (TX; 11 patients: 6 males, 5 females) were performed under general anaesthesia with epidural (EDA). Meanwhile, video-assisted thoracoscopies (VATS; 6 patients: 4 males, 2 females), orthopaedic interventions for total endoprosthesis (TEP) of the hip (10 patients: 4 males, 6 females) and the knee joints (5 patients: all females), as well as unicondylar knee joint prosthesis implantations (5 patients: 1 male, 4 females) were done under general anaesthesia (GA) and parenteral opioid (morphine). Septic patients (11: 5 males, 6 females; mean age of years: 65.6) were treated according to the *Surviving Sepsis Campaign: International guidelines for management of severe sepsis and septic shock: 2008*. Blood samples obtained from healthy volunteers (20

subjects: 12 females, 8 males; mean age of 46.4) after 12 h fasting served as controls and were analysed for comparison. The definition of sepsis was used as it was accepted on the American College of Chest Physicians/Society of Critical Care Medicine consensus conference. (Table 1)

Total number of patients (n)		Type of surgery	Number of patients (n)
Operated:	37	TX:	11
		VATS:	6
		Total hip:	10
		Total knee:	5
		Unicondylar knee:	5
Septic patients:	11		
Healthy volunteers:	20		

Table 1: *The number of patients in the human study (n= 48+20)*

3.1.1.1. Measured molecules in septic patients

The results indicated further investigation of the change of plasma levels of other molecules involved in the pathomechanism in sepsis. The findings confirmed not only the septic state clinically but biochemically as well. The origin of sepsis in patients is in Table 2.

Parallel to the measurement of the plasma level of SOM the level of CRP, PCT, IL-6 and IL-8 molecules was also measured. The level of other molecules which are involved in the pathomechanism of sepsis (CD40, tPA, MCP-1, sP-selectin and VCAM-1) was also detected. As a result of the progression of sepsis there is multi organ failure, which is characterised by respiratory failure. In order to follow MOF the Horowitz score was calculated.

Septic patients, MOF	Number of patients (n)
Ventricular / small bowel perforation, peritonitis	2
Pancreatitis	3
Suture insuff. (due to gastrectomy and Dixon operation)	3
Pneumonia	3

Table 2: *Origin of sepsis on ICU (n=11)*

3.1.1.2. Medication

Inj. Midazolam (0.07 mg/kg, im.) and inj. atropine (0.01 mg/kg, im.) were administered for premedication. The induction of GA was introduced with inj. 1% propofol (1.5–2.5 mg/kg, iv.) and inj. fentanyl (0.0015 mg/kg, iv.). All patients were intubated and ventilated. Muscle relaxation was achieved by giving inj. atracurium (0.5 mg/kg, iv.) at the time of induction; the maintenance dose was 10 μg/20 min, iv. and balanced inhalation anaesthesia was applied (O_2:N_2O in 1:2 volume ratio and sevoflurane in 1.6–2%, v/v). For pain relief inj. morphine (1%; 1–2 mg, iv.) was given in prosthesis and VATS operations as needed, EDA was used in thoracotomies (mixture of inj. bupivacaine 0.25% 20 ml + fentanyl 200 μg + saline 0.9% 26 ml) given as a 6 ml single bolus and continued as needed, 0–10 ml/h). At the end of the operation the effect of the muscle relaxant was suspended by iv. mixture of inj. neostigmine/inj. atropine (2.5 mg/0.5 mg, iv.). Septic patients were intubated under propofol anaesthesia, ventilated and treated under sedation (inj. propofol 1% and inj. morphine 1%, 0–4 ml/h, iv. as needed) at the intensive care unit according to the *"Surviving Sepsis Campaign: International Guidelines, 2008"*.

3.1.1.3. Blood sample taking and radioimmunoassay measurements

In case of thoracic surgery, 10 ml blood samples were taken from the patients 3 times: before the operation, at the time of skin incision or thoracic cavity opening, and at the end of the surgical procedure when the skin was closed and the anaesthesia was terminated. During orthopaedic operations, in addition to the above times a fourth sample was taken directly after removing the damaged tissues and forming the articular surface. In septic patients blood was taken at the time of admission and at 8 am for 4 consecutive days. The previously inserted arterial or venous catheter was used to get the blood samples. All patients were under preoperative fasting (12 h before operation), septic patients had total parenteral nutrition. Blood samples of 10 ml samples were taken and put into ice-cold tubes containing EDTA (Vacutainer) and 200 μl of the peptidase inhibitor aprotinin (Trasylol) was immediately added to each sample to prevent the enzymatic degradation of SOM. After centrifugation at 1000rpm for 5min and then at 4000rpm for 10 min, the plasma was frozen and kept at−20 ◦C until further processing and RIA measurements. SOM-LI was determined by a specific and sensitive radioimmunoassay (RIA) technique developed by the local

laboratory. The sensitivity of this assay is 0.2 fmol/ml, the inter-assay coefficient is 9.2% and the intra-assay variation is 6.4%. C-terminal sensitive somatostatin - 14 antiserum was used which proved to be able to bind both of the biologically active 14 and 28 amino-acid-containing molecular forms of SOM. The peptide was extracted from the plasma by addition of 3 volumes of absolute alcohol. After precipitation and centrifugation (2000rpm for 10 min at 4 ◦C) the samples were dried under nitrogen flow and were re-dissolved in assay buffer before RIA determination. The recovery of this extraction and sample preparation technique is 79.8%.

3.1.1.4. Statistics

Results are expressed as means ± standard errors of mean (SEM). Comparisons between the different data sets of the respective patient groups (before, during and after completing the surgery) and on days 1–4 of septic patients at the intensive care unit compared to healthy volunteers were performed by repeated measures one-way ANOVA and simple one-way ANOVA, respectively, followed by Dunnett's post-test. Probability values $p<0.05$ were accepted as significant.

3.2. Investigation in animal experimental CLP model

3.2.1. Methods and material

3.2.1.1. Resiniferatoxin (RTX)

Resiniferatoxin ($C_{37}H_{40}O_9$) is a natural capsaicin analogue. In order to identify the source of plasma SOM, second group of rats were pretreated with the capsaicin analogue resiniferatoxin (RTX; 30, 70 and 100 µg/kg/day) on 3 consecutive days, 2 weeks before the coecum ligation or sham operation to destroy the capsaicin-sensitive peptidergic sensory nerves. RTX activates the Transient Receptor Potential Vanilloid 1 cation channel on these afferents, which induces a permanent and excessive calcium influx resulting in the destruction of these terminals.

3.2.1.2. „Coecal ligation and puncture" (CLP) method

There are several techniques to induce SIRS in experimental animals. The coecal ligation and puncture (CLP)-induced animal sepsis model shows similar cytokine profile to the human condition, therefore this was used in present study.

We used Wistar type of rats (average weight: 280 ± 11.2 g, average age: 9 ± 0.9 weeks). They were anaesthetised by urethane (1200 mg/kg, im.). We applied 10 rats / group, altogether 3 groups (shame operated in every group). In order to avoid hypoxia we made tracheotomy on each animal and O_2 was given. To maintain normothermia the body temperature was monitored continuously and heating was applied if it was needed.

1. Non-pretreated (intact group)
2. RTX-pretreated (RTX group)
3. Ciclo-somatostatin pretreated (C-SOM group)

In order to identify the source of plasma SOM, second group of rats were pre-treated with the capsaicin analogue resiniferatoxin (RTX; 30, 70 and 100 μg/kg/day) on 3 consecutive days, 2 weeks before the coecum ligation or sham operation to destroy the capsaicin-sensitive peptidergic sensory nerves. In a separate group (3rd group of animals), cyclo-somatostatin (C-SOM; 20 μg/kg) was given antagonizing the effects of SOM at all the five somatostatin receptors was injected i.p. every hour. Sham-operated animals (the same abdominal incision in the same anaesthesia without CLP) served as intact controls in each group. We measured the concentration of SOM in the plasma and in the lung, the MPO activity and examined the mortality / survival effect of SOM.

3.2.1.3. Investigational technique

Six hours after the operation blood was taken by cardiac puncture and centrifuged, the lung was excised, frozen in liquid nitrogen and stored at −70◦C until further processing. SOM-LI immunoreactivity from the plasma and the lung homogenates was determined as described above, myeloperoxidase (MPO) activity of neutrophils and macrophages was determined by spectrophotometry from the lung. After measuring the tissue weight, the lung samples were thawed and chopped into small pieces then homogenized in 4 ml 20 mM potassium-phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 × g at 4◦C for 10 min

and supernatant was removed. The pellet was then re-suspended in 4 ml50 mM potassium-phosphate buffer containing 0.5% hexadecyl-trimethyl-ammonium-bromide (pH 6.0) and centrifuged again. The spectro photometric measurement was done from the supernatant using H_2O_2-3,3_,5,5_-tetramethyl-benzidine (TMB/H_2O_2). Reactions were performed in 96-well microtitre plates in room temperature. The optical density (OD) at 620 nm was measured at the 0 and 5 min. time points using a microplate reader and plotted. The reaction rate was determined as ΔOD/min according to the slope of the line. A calibration curve was then created with the rate of reaction plotted against the human standard MPO preparation. SOM-LI was measured from the supernatant of the homogenates as described for the human plasma.

3.2.1.4. Statistics

Results are expressed as means ± standard errors of mean(SEM). Comparisons between different patient and experimental animal groups were performed by one-way ANOVA followed by Bonferroni's post-test. The survival curves were analyzed with the Gehan–Breslow–Wilcoxon test. In all cases probability values $p < 0.05$ were accepted as significant.

3.3. Results

3.3.1. In clinical investigation

3.3.1.1. Alterations of plasma SOM-LI during thoracic and orthopaedic surgery

The preoperative plasma SOM-LI after 12 h fasting was 7.83±1.41–11.95±1.49 fmol/ml, there was no significant difference between the different groups. In patients undergoing lung surgery, both VATS and thoracotomy significantly increased SOM-LI by 85–88% when the operative procedures were finished and the thoracic cavity was closed (Fig. 1A). In case of orthopaedic interventions, total hip endoprosthesis implantation elevated SOM-LI by 40%, 66% and 80% after skin incision, following the formation of the articular surface and after closing the skin and terminating the anaesthesia at the end of the procedure, respectively. In contrast, neither total, nor unicondylar knee prosthesis operations performed under tourniquet altered SOM-LI in the systemic circulation (Fig. 1B).

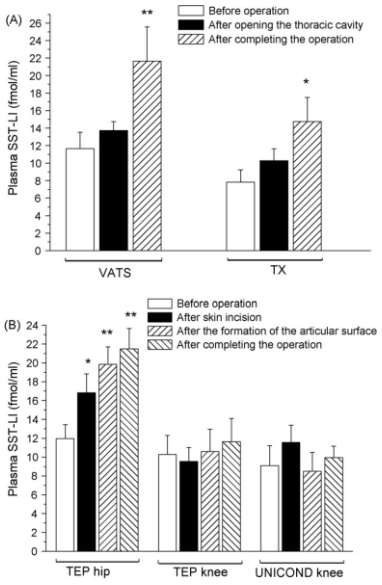

Fig. 1: *Somatostatin-like immunoreactivity (SOM-LI) in the human plasma before, during and after (A) thoracic and (B) orthopaedic surgical interventions. Columns represent means + SEM, *p < 0.05, **p < 0.01 compared to the pre-operation data of the respective patients (repeated measures one-way ANOVA followed by Dunnett's post-test).*

3.3.1.2. Changes of plasma SOM-LI in septic patients

SOM-LI in healthy volunteers was 9.49±0.42 fmol/ml which was similar to the preoperative values of the patients. Compared to these data, the plasma SOM-LI in septic patients at the time of their admission to the intensive care unit before sepsis therapy started was almost 3 times higher. This elevation remained relatively stable throughout the 4 days they spent on the ward and exclusively total parenteral nutrition was given (Fig. 2).

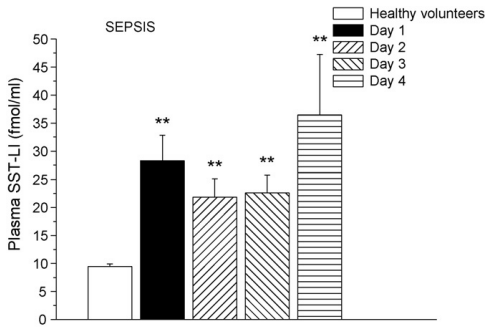

Fig. 2: *Somatostatin-like immunoreactivity (SOM-LI) in the plasma of septic patients. Column represent means +SEM of n=11 patients, *p < 0.01 compared to the data of healthy volunteers (one-way ANOVA followed by Dunnett's post-test).*

3.3.1.3. Changes of plasma level of sepsis markers and Horowitz score

Plasma CRP and PCT also significantly increased, remained at a similar level for 3 days. Their levels remarkably decreased by day 4 as the result of adequate antibiotic treatment and intensive therapy, but still were significantly higher than in healthy volunteers having values below the detection limit. The plasma alterations of CRP and PCT during this early period were very similar. Plasma levels of sP-selectin significantly decreased in septic patients on day 1 compared to healthy control, and it remained relatively stable during the 4 days. The other adhesion molecule, sVCAM-1, significantly elevated in comparison with healthy volunteers having non-detectable values. However, in contrast to sP-selectin, there was a significant decrease by day 3 (Fig. 3).

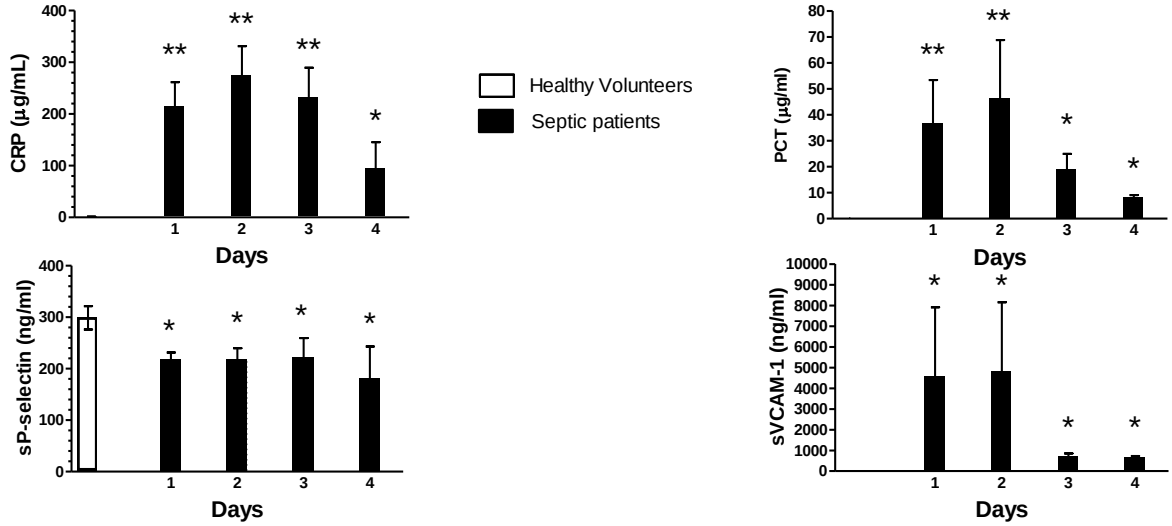

Fig. 3: *C reactive protein (CRP), procalcitonin (PCT), sP-selectin, and sVCAM-1 levels in the plasma of healthy volunteers (n=16) and septic patients (n=11) on days 1-4. Results are expressed as mean ± standard error of mean (SEM), *p < 0.05, **p < 0.001 vs. healthy volunteers (ANOVA + Bonferroni's post test).*

The tPA, as well as the inflammatory cytokines IL-8 and MCP-1, but not IL-6 and sCD40L increased significantly in SIRS patients after admittance. The levels of IL-8 remained elevated throughout the 4 days, but normal tPA and MCP-1 levels were detected on day 4 indicating the effectivity of the therapy, the decrease of the severity of the systemic inflammatory reaction and improvement of the patients' condition. Plasma CD40L only showed a significant elevation on day 2, but it had a great interindividual variation(Fig. 4).

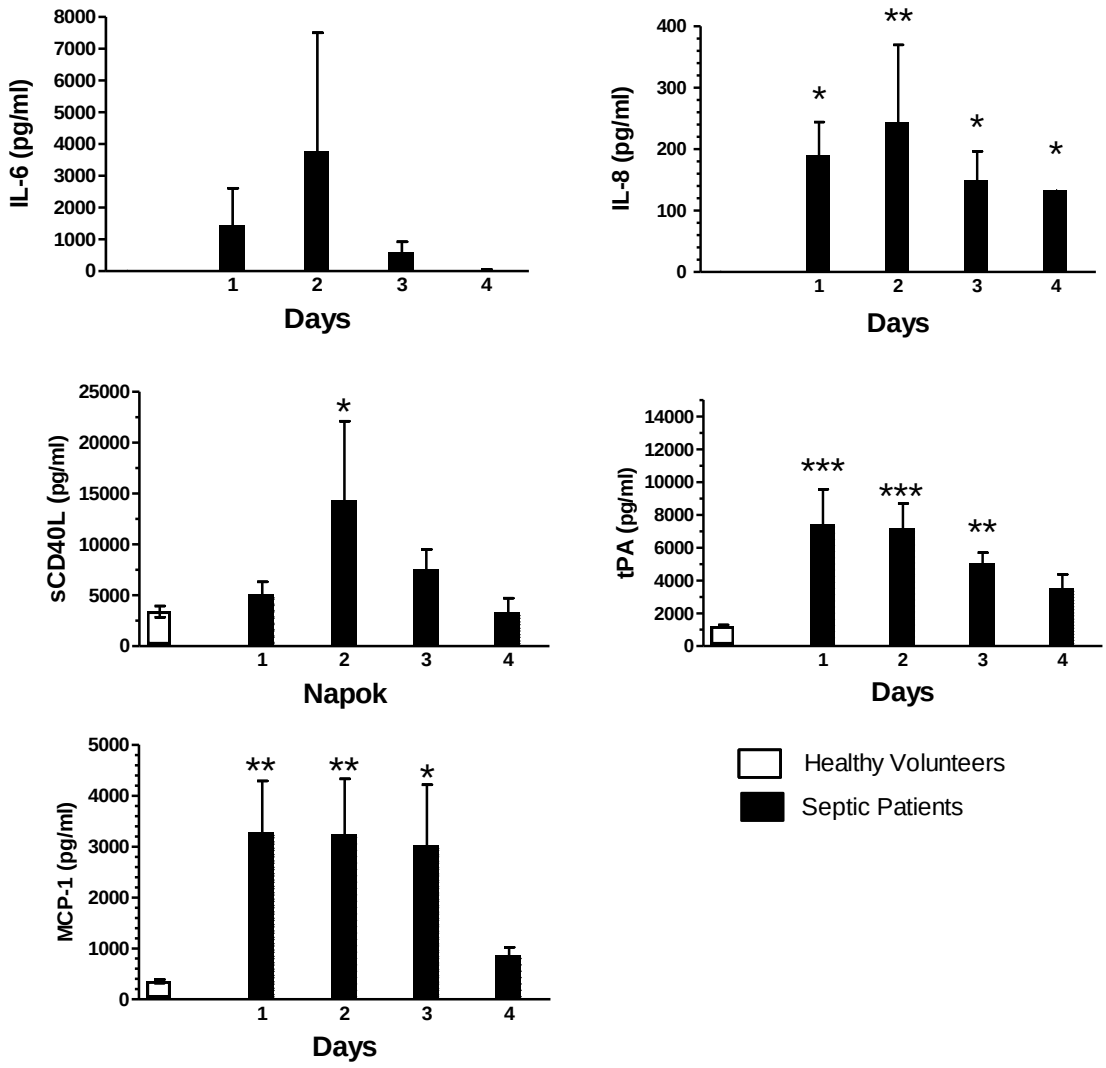

Fig. 4: *IL-6, IL-8, sCD40L, tPA and MCP1 levels in the blood of healthy volunteers (n = 16) and septic patients (n = 11) on the 1st, 2nd, 3rd and 4th days. Results are expressed as mean ± standard error of mean (SEM). *p < 0.05, **p < 0.001 vs. healthy volunteers (ANOVA + Bonferroni's post test).*

The significant decrease of the Horowitz score of these patients throughout this 4-day investigation period confirmed the presence and maintenance of SIRS and severe lung injury, however, there was no significant difference between the day 1 and the day 4 values (Fig. 5).

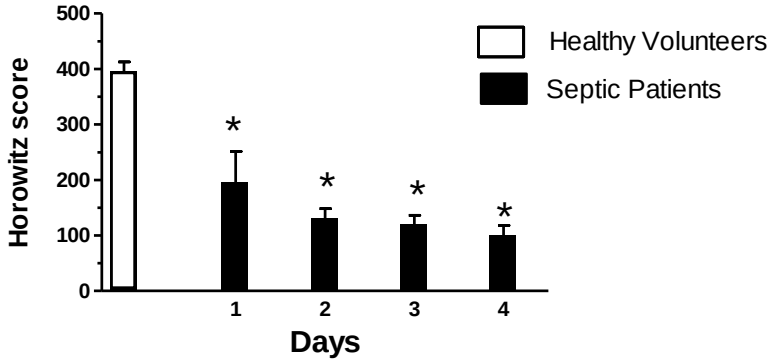

Fig. 5: *Horowitz score of septic patients (n = 11) on the 1st, 2nd, 3rd and 4th days after admittance in comparison with healthy volunteers (n = 16). Results are expressed as mean ± standard error of mean (SEM). *p < 0.0001 vs. healthy volunteers(ANOVA + Bonferroni's post test).*

The symptoms of all patients on day 5 at the time of discharge from the Intensive Care Unit were mild tachycardia and tachypnoe, generalized weakness (loss of body muscle) and lethargy with depression. Blood pressure was maintained, neither vasopressor nor positive inotropic support was required. Renal function was entirely normal or slightly decreased at that time in all patients with acceptable level of urine output. They were breathing spontaneously without additional, extra oxygen requirement at the general ward and they were able to keep oxygen saturation above 90% on 21% of oxygen. Since there were relatively few patients involved in the study with similar symptoms at the time of finishing the intensive therapy, statistical correlation between the physiological parameters(blood pressure, heart rate, respiration rate, saturation, temperature, urine output) and the levels of SOM-LI (or even the other biomarkers) could not be found. Larger number of patients involved in an extensive study is needed to be able to make subpopulation analysis and reveal potential correlations.

3.3.2. In animal CLP model

3.3.2.1. Plasma SOM-LI change in animal CLP model

There was a significant, 2-fold and 1.5-fold elevation of SOM-LI in the plasma and the lung, respectively 6 h after coecum perforation as compared to the sham operated (intact) rats. Following RTX pre-treatment, when the capsaicin-sensitive peptidergic primary sensory neurones were defunctionalized, the SOM-LI was not altered in the intact, non septic animals, but the SIRS-induced elevation was absent both in the plasma and the lung (Fig. 6).MPO activity in the lung showing neutrophil and macrophage accumulation, as an important and sensitive marker of the inflammatory reaction, significantly increased 6 h after the operation compared to the intact rats. Interestingly, this value was remarkably higher, almost double in the lung of the sham-operated animals after RTX-desensitization, which did not increase further after the coecum perforation. In the C-SOM-treated group, the basal MPO activity in the lung of the sham-operated rats was similar to the non-treated ones, but it was significantly greater 6 h following CLP (Fig. 6).

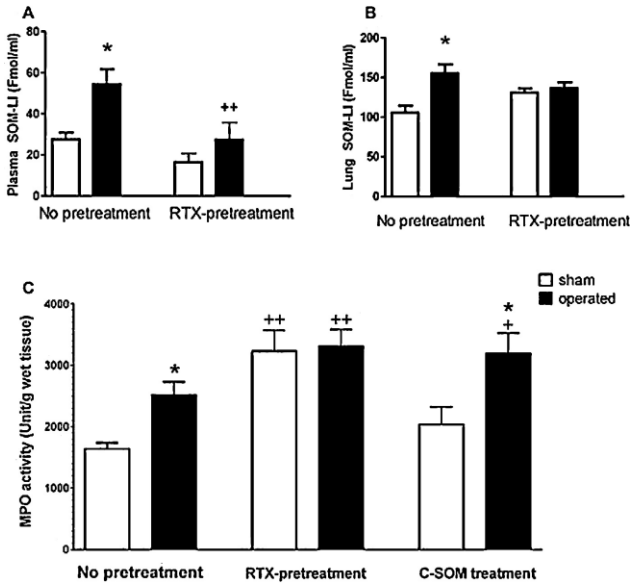

Fig. 6: *Somatostatin-like immunoreactivity (SOM-LI) of the (A) plasma and (B) lung of non-pretreated, resiniferatoxin (RTX)-pretreated, as well as cyclo-somatostatin (C-SOM)-treated intact (sham-operated) and operated (coecum perforation) rats 6 h after the procedure (n=5–12/group). Panel C shows the myeloperoxidase activity (MPO) of the lung of the same animals. Each column represents the mean ± standard error of mean (SEM), *p < 0.05 vs. respective intact group,* $^{++}$*p < 0.01 vs. respective non-pretreated rats (ANOVA + Bonferroni's post test).*

3.3.2.2. Mortality in none pretreatment, RTX-pretreatment and C-SOM treatment groups

Most of the animals survived the 6 h of the experiment in the non-pretreated groups, but after the defunctionalisation of the capsaicin-sensitive afferents with RTX pre-treatment 40% of the rats died in the first hour and an additional 20% in the second hour showing a significantly decreased survival. In the group repeatedly treated with C-SOM during the 6-h experiment, a decreased survival tendency was observed as compared to the saline-treated SIRS rats, but the difference was not statistically significant (Fig. 7).

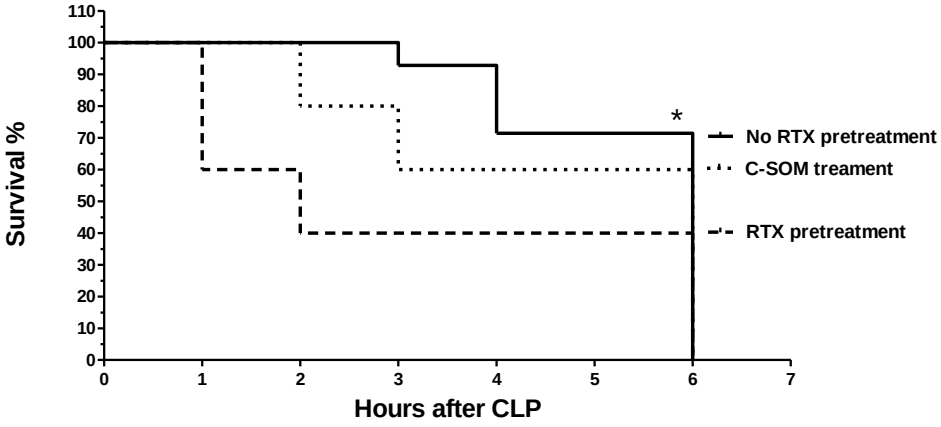

Fig. 7: *The percentage of survival of non-pretreated, resiniferatoxin (RTX)-pretreated and cyclo-somatostatin (C-SOM)-treated rats during the 6 h after coecum puncture,*p < 0.05 (Gehan–Breslow–Wilcoxon test).*

4. Discussion

4.1. Change of plasma somatostatin concentration in patients during surgery

The present results provide evidence for a significant increase of plasma SOM-LI during the two types of thoracic surgical procedures performed under general anaesthesia. Several previous data obtained in animal experiments suggests that stimulation of sensory nerves during surgery, even under moderate invasive conditions such as VATS, can induce SOM release resulting in the rise of its plasma level. In case of both types of thoracic surgery, a significant, about 85–88% increase of plasma SOM concentration was observed directly after completing the operation, closing the thoracic cavity and the skin, and terminating anaesthesia. Despite the smaller size of incision and tissue damage in the VATS group, both

the maximal elevation and the post-operative level of plasma SOM-LI were non significantly, but higher in patients having this type of surgery than thoracotomy. The reason for this observation might be the different types of analgesia we administered during surgery. All opioids are known to reduce the release of sensory neuropeptides, including somatostatin, from sensory nerve terminals which is also likely to be involved in our results. In patients with orthopaedic surgery, the extent of plasma SOM-LI elevation (80%) was similar in case of hip endoprosthesis to that observed in the thoracic surgery groups. However, there was a significant increase after skin incision and the elevated SOM level could be observed throughout the surgical procedure. This latter finding might be due to the fact that total hip prosthesis operation is one of the most painful types of surgical interventions. In contrast to the hip operations, plasma SOM levels were not altered in patients with either type of knee prosthesis (total and unicondylar) surgeries. These interventions were done under tourniquet, therefore, the locally released SOM presumably could not reach the systemic circulation. Noxious interventions and tissue damage during lung or joint surgery, such as pulling and cutting the pleura, the lung parenchyma, the joint capsule or the synovium activate peptidergic sensory nerves. The higher SOM-LI elevation in the present operative procedures might be explained by the greater tissue damage and more intensive stimulation of the nociceptive afferents in cases of thoracic and orthopedic surgeries. In septic patients the SOM level in the systemic circulation was significantly higher at the time of admission and throughout the 4-day examination period than in normal population. This remarkable, about 2–3-fold elevation is likely to be due to the severe ongoing inflammatory process, in response to which a variety of inflammatory mediators are produced throughout the body. Leukotriens, protons, prostaglandins, bradykinin and inflammatory cytokines are able to activate and/or sensitize peptidergic afferents from which SOM is released. Somatostatin exists in 14 and 28 amino-acid-containing forms in the plasma, the specific and sensitive RIA technique developed in our laboratories measures both. However, there is molecular pharmacological evidence that human sst_4 receptors exhibit specific binding to SOM- 14. Therefore, we suppose that 14 amino-acid forms might have particularly important role in the mediation of the endogenous anti-inflammatory and analgesic actions.

In conclusion, this human study provided evidence that thoracic and orthopaedic surgical interventions increase SOM-LI in the plasma. It is assumed on the basis of our earlier animal

experiments that SOM is released from the sensory nerves activated by tissue damage and inflammatory mediators, and it reaches the systemic circulation. Since this neural somatostatin-mediated endogenous anti-nociceptive and anti-inflammatory “sensocrine” system has already been established in laboratory animals, it can be assumed that a similar mechanism might also operate under human pathophysiological conditions.

4.2. Change of plasma somatostatin concentration in septic patients and experimental animal CLP model

The present clinical results provide clear evidence for a significant and long-lasting increase of plasma SOM-LI in response to well-established severe systemic inflammatory reactions supported by a variety of conventional laboratory parameters, as well as specific inflammatory cytokines and adhesion molecules. SOM-LI remained elevated at the same level, which was approximately 3 times higher than the basal values measured in healthy volunteers, during the 4 days of the intensive care treatment. Then the patients’ general clinical condition remarkably improved as a result of the combined antibiotic and other conventional intensive therapy and they were discharged from the Intensive Care Unit. Lung damage was confirmed by the routinely used Horowitz score decrease, and the systemic inflammatory reaction with the increase of the sensitive laboratory markers CRP and PCT. These latter parameters decreased by the end of the 4-day investigation period, but were still elevated compared to healthy volunteers. Since the host defence systems rather than the invading pathogens are responsible for the multi organ failure in SIRS, the activation of the coagulation with concurrent down-regulation of the anticoagulation system and fibrinolysis is important in the progression. Inflammation-induced endothelial dysfunction and intravascular coagulation contributes to further triggering of the inflammatory pathways. Pro-inflammatory cytokines and other mediators activate the coagulation system and inhibit the important physiological anticoagulant pathways through an IL-6-induced expression of tissue factor on activated mononuclear cell sand endothelial cells and are insufficiently counteracted by physiological anticoagulant mechanisms and endogenous fibrinolysis. Researchers have recently revealed that sepsis markedly increases platelet adhesion, fibrin deposition and capillary thrombosis that requires platelets, P-selectin and coagulation activation. In a very recent paper, VCAM-1, as an endothelial biomarker, was

found to be significantly associated with sepsis severity. Our data showed a clear decreasing tendency of this soluble molecule by day 3 suggesting that the intensive treatment had slowed down the progression of endothelial dysfunction. The additional novel, important and interesting points of our study is that we showed an elevation of the soluble VCAM-1 and a significant decrease of sP-selectin in the peripheral circulation of these septic patients. The remarkably lower level of P-selectin was not altered during the 4 days, just like the elevation of SOM-LI. Increase of its plasma concentrations has been shown in peripheral arterial occlusive disease and atherosclerotic conditions, but present study is the first to show its decrease in sepsis. However, the functional relevance and explanation of these findings need further investigations. The fibrinolytic molecule tPA plays a key role in the complex pathophysiological mechanisms of SIRS, its exogenous administration even worsens the short-term functional outcomes and data of present study support this finding. Similarly to MCP-1, tPA elevation also decreased to the level of healthy subjects by day 4 in our patients showing that the function of the anticoagulation system normalized by then. IL-6 and IL-8 are both synthesized by activated macrophages and promote their recruitment and accumulation. Although the plasma concentrations of IL-8 were much lower than those of IL-6, it had a significant elevation during the total investigation period. This is in agreement with what was found earlier by other groups in septic patients. In SIRS and sepsis IL-6 is one of the most widely studied cytokines. Its plasma level has been shown to increase due to inflammation/infection (tissue damage like trauma, burns, sepsis). Despite the great absolute values, the alterations found in our patients did not prove to be statistically significant due to the great inter-individual variations presumably due to the divergent regulation of its expression. Therefore, it is assumed that this is not the crucial patho-physiological factor and key regulator of SIRS. $CD4^+$ T-cells are activated by IL-6 and they produce sCD40L, which activates endothelial cells resulting in more cytokine and chemokine production. Its interaction with CD40 localized on smooth muscle and endothelial cells triggers the release of inflammatory mediators, which activates the coagulation cascade by increase the activity of metalloproteinases. Its plasma concentration was significantly elevated only on day 2, showing that platelet activation is not the earliest step in SIRS, but then its decrease indicates the effective therapy. The role of sCD40L is strengthened by the finding that its level is elevated in patient with SIRS. MCP-1 is produced by activated

macrophages and is responsible for the recruitment of memory T, dendritic cells and monocytes to the place and location of inflammation, infection and injury. Present results showed its significant increase in the plasma of SIRS patients similarly to others, but it went back to the normal level by day 4 when they were discharged from the Intensive Care Unit showing that it sensitively correlates with the clinical picture.

It has been established by our previous studies that the broad range of anti-inflammatory effects of somatostatin are predominantly mediated by the somatostatin sst_1 and sst_4 receptors. SOM exists in 14 and 28 amino-acid containing forms throughout the body including the plasma. Human sst_4 receptors expressed by COS-7 cells exhibit specific preference to bind SOM-14, therefore, this can be supposed to have a particularly important role as an endogenous anti-inflammatory mediator. It has been proved here that a remarkable plasma SOM-LI elevation occurs in the widely used rat model of SIRS similarly to the human condition, so this is a general counter-regulatory mechanism. In the animal experiments after destroying these capsaicin-sensitive peptidergic sensory fibres with RTX pretreatment the plasma SOM concentration elevation was absent, the neutrophil/macrophage accumulation shown by the MPO activity was greater, and the survival was significantly worse. C-SOM is the only available, but relatively weak somatostatin receptor antagonist blocking its effect at all the five cloned sst receptors. Its repeated administration every hour during the 6-h investigation period significantly increased the SIRS-induced elevation of MPO in the lung as compared to the saline-treated CLP group similarly to the RTX pre-treatment. The non-significant survival decreasing tendency might be explained by the weak and non-selective antagonism of the sst receptors. These results provide evidence that SOM is released from the sensory nerves and it has a protective role in the systemic inflammatory reaction.

This is the first comprehensive study in humans and animal experiments providing evidence that SOM is released from the peptidergic sensory nerves in response to activation by a variety of inflammatory mediators. It gets into the bloodstream and mediates a potent endogenous protective mechanism, which decreases mortality.

5. Theses

1. There is a significant increase of the level of somatostatin in the plasma in case of thoracotomies (TX and VATS) and in orthopaedic surgery (total hip and knee prosthesis, unicondylaris knee prosthesis operation) during surgery and thereafter as well.
2. The somatostatin is released locally from the vesicles of the sensory nerves. From this place the somatostatin enters to the systemic circulation.
3. The measured change in the concentration of somatostatin is influenced by the type of surgery, the degree of tissue damage and the applied pain controlled method.
4. According to our clinical and animal experimental models we can say that the concentration of plasma somatostatin is significantly increased in systemic inflammatory response syndrome in sepsis.
5. In our experimental animal CLP model we proved that the somatostatin is released locally from the sensory nerve terminals and enters the systemic circulation. This sensory type somatostatin is able to reduce mortality and inflammatory reaction, improves survival.
6. According to our results it can be concluded that the somatostatin released in the sensory nerve terminals by pain stimulation and systemic inflammatory response syndrome reaction has endogenous pain reducing and anti-inflammatory effect.

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