Regulated upon activation, normal T-cell expressed and secreted chemokine and interleukin-6 in rheumatic pulmonary hypertension, targets for therapeutic decisions

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Abstract

Background: Recent studies have highlighted the possible influence of chemokines and cytokines on several types of pulmonary arterial hypertension (PAH). Increasing interest has also been focussed on their role as a cause of post-cardiopulmonary bypass (CPB) organ dysfunction.

Hypothesis: Chemokines and cytokines are involved in the pathobiology of rheumatic PAH.

Methods: Serum levels of the chemokine, regulated upon activation, normal T-cell expressed and secreted (RANTES) and the cytokine interleukin-6 (IL-6) were measured by enzyme-linked immunosorbent assay (ELISA) in 35 patients with rheumatic mitral valve disease and 10 matched healthy subjects (control group). Eleven patients (31.4%) had severe pulmonary hypertension. Subsequently, 23 patients underwent mitral valve replacement. The relation of RANTES and IL-6 circulating level with postoperative organ dysfunction was analysed through multiple organ dysfunction score (MODS).

Results: Patients with severe PAH have a significantly higher mean serum level of RANTES compared with other patients (6138.6±3543.8 pg/ml vs 1818.2±475.2 pg/ml, p=0.0003). The serum level of IL-6 in the patients was statistically different from that of the control (378±50.8 pg/ml vs 262±90.5 pg/ml, respectively, p=0.002). Patients who required postoperative inotropes had higher preoperative and post-CPB levels of both RANTES and IL-6. While patients with postoperative lung dysfunction had higher levels of IL-6 preoperatively and post-CPB and lower levels of RANTES post-CPB.

Conclusions: RANTES and IL-6 should be investigated as potential therapeutic targets in the control of rheumatic PAH. Improved understanding of the contribution of RANTES and IL-6 to adverse postoperative complications can lead to improved patient outcome.

Keywords: Rheumatic; Pulmonary hypertension; RANTES; IL-6; Organ dysfunction

1. Introduction

Pulmonary arterial hypertension (PAH) is a frequent serious complication of rheumatic mitral valve disease (MVD) in Egypt. Data from previous studies suggested that at least three mechanisms contribute to development of pulmonary hypertension in MVD: passive transmission of elevated left atrial pressure, reactive pulmonary arteriolar vasoconstriction and morphologic changes in pulmonary vasculature [1].

Recent discovery that primary pulmonary hypertension (PPH) can be associated with production of several cytokines and growth factors, including interleukin-1 (IL-1), IL-6 and chemokines, including regulated upon activation, normal T-cell expressed and secreted (RANTES), highlighted the possible influence of inflammatory mechanisms in this condition [2,3]. However, data regarding PAH in rheumatic heart disease (RHD) are lacking.

PAH significantly influences the clinical findings and prognosis, and not surprisingly, the impact of PAH on morbidity and mortality is highly dependent on its degree of severity. Severe PAH is associated with a high risk of perioperative morbidity and mortality (10—15%) in patients undergoing mitral valve replacement (MVR) as well as with increased mortality in the long term [4].
Earlier studies have demonstrated that cardiopulmonary bypass (CPB) activates several inflammatory pathways with subsequent release of several mediators including cytokines, such as IL-1, IL-6 [5], and chemokines, such as monocyte chemoattractant protein-1 (MCP-1) and RANTES [6]. Increasing interest has been focussed on their role as a cause of postoperative organ dysfunction described in patients undergoing open heart surgery [7].

Chemokines are a group of chemotactic cytokines promoting attraction and activation of granulocytes, monocytes and lymphocytes [3]. They guide leucocytes through the endothelial junctions and the underlying tissue and activate leucocyte effector functions [8].

2. Aim of the work

This study aimed to investigate the circulating level of the chemokine RANTES and the cytokine IL-6 in patients with rheumatic MVD associated with PAH. We also investigated their possible relation to postoperative organ dysfunction.

3. Patients and methods

3.1. Subjects

The patients recruited for this prospective study were 35 adult patients with rheumatic MVD (24 females and 11 males, mean age 31.8 ± 9.7 years, range: 18–55 years). Ten matched healthy subjects (five females and five males, mean age: 32.7 ± 10.2 years, range: 20–52 years) served as a control group. Subsequently, 23 patients who were candidates for open heart surgery underwent MVR at the Cardiothoracic Surgery Department, Tanta University, Egypt.

Clinical characteristics including demographics, medical history and echocardiographic data were recorded for all patients.

3.2. Echocardiography

Two-dimensional (2D) echocardiographic assessment of the mitral valve morphology was done with characterisation of the severity and extent of the pathological process. Doppler echocardiographic analysis also yielded important information concerning mitral regurgitation, aortic and tricuspid valves.

3.2.1. Pulmonary artery pressure (PAP) measurements

Mean maximum tricuspid regurgitation (TR) velocity of three waveforms was recorded in milliseconds and inserted into the modified Bernoulli equation \( (4V^2) \); a fixed value of 10 mmHg (right atrial pressure) was added to yield systolic PAP [9].

Mild pulmonary hypertension was defined as \( PAP < 40 \) mmHg, moderate pulmonary hypertension as \( PAP = 51–75 \) mmHg and severe pulmonary hypertension \( PAP > 75 \) mmHg [10].

3.3. Intra-operative management

Anaesthesia protocol was the same for all patients. The conduct of CPB was similar in all patients using Ringer’s lactate as the priming solution. Haematocrit was maintained at about 24. Core cooling was instituted until core temperature was reduced to 28–30 °C, and then cold (4 °C) crystalloid antegrade cardioplegia was infused. MVR was done using a standard technique. No modified ultrafiltration or leucocyte depletion techniques were employed for any of the patients throughout the study period.

3.4. Collection of samples

A venous blood sample was collected from the healthy subjects (control group) and all the patients. A complete blood picture was also obtained. In patients undergoing surgery, serial blood samples were collected from the arterial line before and after induction of anaesthesia, at 15 min after the onset of CPB, at the cessation of CPB and at 24 h after the cessation of bypass while the patient was still in the intensive care unit (ICU). The serum was separated and kept at −20 °C until the measurement of both RANTES and IL-6 level was done.

3.5. Measurement of serum level of RANTES and IL-6

Commercial enzyme-linked immunosorbent assay (ELISA) kits for both RANTES (Bender MedSystems, Inc., USA) and IL-6 (R & D Systems, Inc., USA) were purchased, and testing was performed according to the manufacturers’ instructions.

3.6. Postoperative data

Postoperative data, including ventilator status, blood gas variables, inotropic support, blood picture, serum urea and creatinine level, were collected. Global and specific organ dysfunctions were assessed with the multiple organ dysfunction score (MODS) [11] after surgery.

3.7. Statistical analysis

Data are shown as means ± standard deviation (SD) and percentage. Differences between groups were analysed with Mann–Whitney U test for independent samples and Wilcoxon test for dependent paired samples. For the assessment of sequential variations in circulating levels of the markers studied, we used an analysis of variance for repeated measures with Friedman test. Values of \( p < 0.05 \) were considered statistically significant.

4. Results

Echocardiographic data of our patients are summarised in Table 1. Variable degrees of PAH were present in all patients with severe pulmonary hypertension in only 11/35 (31.4%) of patients.
4.1. Serum level of RANTES in the control and patient groups (Table 2)

The venous serum level of RANTES in patients was significantly higher than in the control group \((p = 0.009)\). However, rheumatic patients with severe PAH have a mean serum level of RANTES of 6138.6 ± 3543.8 pg/ml, which is significantly greater than that of patients without severe PAH (1818.2 ± 475.2 pg/ml) \((p = 0.0003)\).

4.2. Serum level of IL-6 in the control and patient groups (Table 2)

The patients’ serum level of IL-6 was statistically different from that of the control group (378 ± 50.8 vs 262 ± 90.5, respectively, \(p = 0.002\)). Comparison of IL-6 serum level in patients with and without severe PAH showed that the level is higher in patients with severe PAH but without statistical significance \((p = 0.08)\).

4.3. Haematological results of the control and patient groups

There was no statistically significant difference between the control and the patient groups regarding total leucocytic, neutrophil and platelets count. The significant difference observed in differential leucocytic count was a higher monocyte and lymphocyte count in patients than in the control group \((p = 0.003, p = 0.02\), respectively). This significant difference has been also demonstrated in patients with severe PAH in comparison to other patients (Table 2).

4.4. Pattern of serum RANTES circulating level in patients undergoing MVR

The mean preoperative RANTES level of 3520 ± 2811.3 pg/ml was significantly raised after induction of anaesthesia (4040 ± 3161.2 pg/ml) \((p < 0.0001)\), and then there was a significant decrease after initiation of CPB, with values reaching 2493 ± 2087.4 pg/ml \((p < 0.0001)\). At the end of CPB, the RANTES level increased significantly towards the preoperative level \((p < 0.0001)\) and remained stable over 24 h (3060 ± 1311.8 pg/ml) \((p = 0.17)\) (Fig. 1). The significant sequential variations in circulating levels of RANTES were clearly demonstrated by Friedman test \((p < 0.0001)\).

4.5. Pattern of serum IL-6 circulating level in patients undergoing MVR

The mean arterial IL-6 serum level before the operation was 388.6 ± 46.4 pg/ml, and it began to rise after induction of anaesthesia (474.1 ± 78.4 pg/ml) \((p < 0.0001)\) but showed a decrease after institution of CPB \((p = 0.002)\).
The level reached a maximal value at the end of the CPB to 785.9 ± 113.9 pg/ml (p < 0.0001). The level gradually declined but it remained elevated for 24 h thereafter (p < 0.0001) (Fig. 2). These significant sequential variations in circulating levels of IL-6 were also demonstrated by Friedman test (p < 0.0001).

4.6. Postoperative organ dysfunction

Of our 23 patients, 12 (52.17%) had one or more postoperative organ dysfunction according to MODS. The mean MODS was 2.4 ± 0.6. Of the 23 patients, eight (34.8%) required inotropes (seven of them had severe PAH), five (21.7%) had lung dysfunction (three of them had severe PAH) and three (13%) had renal dysfunction (all had severe PAH). Only seven patients (30.4%) had postoperative haematological dysfunction.

4.7. Relation of RANTES and IL-6 circulating level to postoperative organ dysfunction

The mean serum concentration of RANTES was significantly higher after anaesthesia in patients requiring inotropes than those who did not require support (p < 0.0001). While it was significantly lower at the end of CPB in patients with lung dysfunction (p = 0.0007), the mean IL-6 serum concentration was significantly higher after CPB in patients with postoperative lung dysfunction (p = 0.02) and in patients requiring inotropes (p = 0.03). As regards postoperative renal dysfunction, no statistical difference in serum concentration of RANTES and IL-6 could be achieved between patients with and without renal dysfunction (Figs. 3 and 4).

5. Discussion

Earlier studies have associated sustained high levels of inflammatory markers with pathogenesis of PAH, poor outcomes in systemic inflammatory response syndrome and in patients undergoing open heart surgery [5,12]. These markers are therefore of diagnostic and therapeutic interest. Our results demonstrated an altered cytokine and chemokine milieu of the peripheral blood in rheumatic patients, especially with severe PAH. In this study, one of the main finding was that the mean RANTES circulating level was significantly higher in RHD patients with severe PAH than in other patients. The relation of RANTES to PPH has been investigated extensively in many studies [13]. It has been shown previously that the chemokine RANTES is an important chemoattractant for monocytes and lymphocytes [3] that represent a significant portion of cell population within the perivascular infiltrates of PPH.

This may be relevant to our finding of significantly higher lymphocyte and monocyte count in the group of patients with severe PHT. We believe this could be related to increased serum level of RANTES in this group of patients. However, the possibility of finding different subtypes of monocytes in different types of PHT has been raised by Bull et al. in 2004.

It is worthy of mention that the present data demonstrated significant changes in the circulating levels of the chemokine RANTES and the cytokine IL-6 after CPB in rheumatic adult patients. Specific organ dysfunctions had relations with chemokine and cytokine level at different times. Generally, the patients with lung dysfunction had higher levels of IL-6 and lower levels of RANTES at the end of CPB while the patients requiring inotropes had higher levels of both IL-6 and RANTES before and after CPB.

The association of lower RANTES level after CPB with complicated clinical course has been reported previously in a study including children with congenital heart disease who were subjected to corrective surgery with deep hypothermic arrest [6]. Our results are contradictory regarding a higher pre- and post-CPB RANTES level in patients requiring postoperative inotropes. We believe that this difference is due to the different patient population and pathological process. This observation reinforces the putative role of this regulatory chemokine in myocardium injury in RHD. The differential cytokine polarisation in the heart in RHD is probably related to immigrant autoreactive T cells, local chemokines produced by inflammatory cells and adhesion molecules. Blocking studies showed that the capture of T cell was mediated by TNF-induced RANTES [20]. In another animal model of destructive autoimmune myocarditis, certain chemokines, including macrophage inflammatory protein-1 (MIP-1) and RANTES, polarised the recruitment of T-helper (Th1 and Th2) subsets [21].

Serum IL-6 concentration seems to be a good indicator of activation of the inflammatory cascade and predictor of subsequent organ dysfunction. Our findings of high IL-6 serum level in patients with complicated postoperative course compared with those with uneventful ones are consistent with the reports of others [22,23].

Raised IL-6 levels have been reported to correlate with post-CPB left ventricular dysfunction and ischemic episodes [22]. In addition, Halter et al. [23] demonstrated that increased IL-6 levels at the end of CPB correlate with reduced lung function postoperatively.  

5.1. Limitations of the study

This study is concerned with detection of circulating levels of cytokines and chemokines; however, previous results suggested an important role of inflammatory mediators synthesised by resident cells in specific organs in the evolution of the inflammatory response [24].

The volume of priming solution in CPB can be seen as a limitation because of its haemodiluting effect [25]. This could underestimate the early postoperative values of circulating levels of cytokines.

Another factor that may influence the degree of the inflammatory response is the individual-specific reactivity of the cytokine system. This has not yet been fully characterised. In 1993, Ribeiro et al. [1] in their study on 100 patients with MS, showed that different pulmonary vascular dynamics exist in patients with similar degree of left atrial pressure. This indicated that individual or genetic background may ultimately determine the PAP response to the disease.
6. Conclusions

Despite of the multifactorial nature and complex mechanisms of pulmonary hypertension in RHD, RANTES and IL-6 should be investigated as potential therapeutic targets in the control of rheumatic PAH. Improved understanding of the contribution of the chemokine RANTES and the cytokine IL-6 to adverse postoperative complications can lead to improved patient outcome.

References