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BRIEF REPORT

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Circulating interleukin-1 receptor antagonist (IL-1RA) serum levels in patients undergoing orthotopic heart transplantation

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Abstract In a pilot study we determined the serum levels of circulating interleukin-1 receptor antagonist (IL-1ra) in patients undergoing orthotopic heart transplantation and in control patients scheduled for open heart surgery without allograft transplantation. Blood samples were obtained from 12 transplant recipients and 7 controls prior to the operative procedures to determine baseline values. Serum levels of IL-1ra were measured within 12 h of decrossclamping of the aorta and every 24 h for the following 14 days. Endomyocardial biopsies were obtained weekly for the 1st month after transplantation. Compared to baseline values, IL-1ra serum levels 12 h after decrossclamping of the aorta were significantly higher both in the control group (507 ± 165 vs 3980 ± 452 pg/ml, $P < 0.01$) and among the transplant recipients (413 ± 180 vs 4117 ± 459 pg/ml, $P < 0.01$) IL-1ra levels remained significantly elevated for 2 and 5 days, respectively. There were no significant differences in the IL-1ra serum levels between the two groups throughout the observation period. Endomyocardial biopsies of two patients showed acute allograft rejection, Billingham grade III a and III b, respectively. In both cases, the rejection episodes were accompanied by a renewed and more pronounced elevation in the IL-1ra serum levels beyond 4000 pg/ml for at least 2 days. These preliminary results indicate that IL-1ra may be a nonspecific immune marker during the first few days after orthotopic heart transplantation and cardiopulmonary bypass. Moreover, renewed, prolonged increases in IL-1ra appear to be associated with rejection. Further studies are needed to confirm the predictive value of IL-1ra in the detection of acute allograft rejection.

Key words Interleukin-1 receptor antagonist, heart transplantation, allograft rejection · Heart transplantation, interleukin-1 receptor antagonist · Allograft rejection, interleukin-1 receptor antagonist, heart transplantation

Introduction

Interleukin-1 (IL-1) is the term used to refer to two polypeptides that possess a wide spectrum of inflammatory, metabolic, hematopoietic, and immunological properties. Although IL-1 and IL-1β are distinct gene products, they share various biological activities because they recognize the same cell surface receptors. In the early 1980s, specific IL-1 inhibitors were described [7]. The IL-1 receptor antagonist (IL-1ra) is a naturally occurring human protein that binds to both types of IL-1 receptors. It sterically inhibits IL-1 from binding and thereby disrupts signal transduction and cellular responses induced by both types of IL-1 [2]. IL-1ra is able

to attenuate the cytokine cascade [10], and promising results have been obtained using IL-1ra for the treatment of sepsis [9].

Because IL-1 plays a pivotal role in immune nonself recognition, experimental studies have been performed to evaluate IL-1ra as an adjunct to immunosuppressive therapy. Shiraishi et al. demonstrated the effectiveness of IL-1ra in combination with low-dose cyclosporin in reducing the inflammatory response and rejection after organ transplantation [18]. Immunoregulatory cytokines have been implicated in the pathophysiology of allograft dysfunction and rejection [6]. However, little is known about the pattern of IL-1ra serum levels in patients with allografts. Following renal transplantation, Daniel et al. included the determination of IL-1ra serum levels in their cytokine monitoring studies in order to differentiate between immune activation due to infection and that due to rejection [4]. Values above a cut-off value of 4200 pg/ml of the circulating IL-1ra serum levels were well correlated with graft rejection. To our knowledge, IL-1ra serum levels after orthotopic heart transplantation have not been assessed. Hence, the aim of the present study was (1) to determine the pattern of IL-1ra serum levels early after orthotopic heart transplantation and (2) to examine a possible correlation between IL-1ra serum levels and histological criteria of graft rejection. In addition, a control group of patients was studied in order to evaluate IL-1ra serum levels in patients undergoing cardiopulmonary bypass without allograft transplantation.

Material and methods

Patients

Nineteen consecutive adult patients undergoing orthotopic heart transplantation ($n = 12$) or other cardiac operations with cardiopulmonary bypass ($n = 7$) at our clinic within a 1-year period were studied. Patients with clinical signs of infection after transplantation, i.e., leukocyte count > 10,000/μl and body temperature > 38.5 °C, were excluded. Patients requiring excessive blood transfusions due to postoperative bleeding were also excluded from the study. Twelve patients with an uneventful postoperative course after heart transplantation were enrolled in the study.

A control group of seven adult patients undergoing cardiopulmonary bypass surgery during the same period was studied. The six patients who had coronary bypass grafting and the one who had aortic valve replacement were comparable in terms of the time of crossclamping and the duration of the operative procedure. Patients with infection or excessive blood transfusions were also excluded from the controls.

Cardiopulmonary bypass

Cardiopulmonary bypass was established with moderate to mild systemic hypothermia (26°–30 °C) with flow rates of 2.0–2.4 l × min^{-1} × m^{-2} and a mean arterial pressure of 40–50 mm Hg. A left ventricular vent was placed through the right pulmonary vein. Crystalloid cold cardioplegic solution was flushed and the heart was externally cooled with cold (4 °C) saline solution. This was repeated, if necessary, to ensure the absence of ventricular electrocardiographic or mechanical activity throughout the operation. Cardiopulmonary bypass was not discontinued until the rectal temperature had reached 35 °C. Prior to heart transplantation, the recipient heart was explanted without the application of crystalloid cardioplegic solution. The donor heart was initially perfused with 3000 ml of ice-cold HTK solution and then stored at 4 °C in cold HTK solution and external ice.

Blood sampling for IL-1ra measurement

Samples were drawn from the central venous line. They were collected in plastic tubes and centrifuged immediately at 20 °C at 3000 rpm for 10 min. The serum was aliquoted into sterile tubes and stored at –70 °C until measurement. After obtaining informed consent, blood samples were collected 2 h prior to the operative procedure, 12 h after decrossclamping of the aorta, and every 24 h thereafter for 14 days. For patients undergoing heart transplantation, blood samples were drawn every morning prior to the first daily oral or intravenous administration of immunosuppressive agents.

Immunosuppressive protocol

The immunosuppressive protocol consisted of induction therapy with antithymocyte globulin, which was started within 8 h of transplantation and continued until day 6, 1 g of methylprednisolone 1 h before transplantation and 1 g just prior to decrossclamping of the aorta, followed by 500 mg within 12 h and 250 mg within the following 24 h. Beginning on the 2nd day post-transplantation, patients received 1 mg/kg per day of prednisolone, followed by a tapering protocol reducing prednisolone by 5 mg every 2nd day until a final dose of 0.1–0.3 mg/kg per day was reached. Cyclosporin was given in a dose of 1–6 mg/kg per day to reach a target serum level of 300 ng/ml (Abbot TDX whole-blood fluorescent assay), and azathioprine in a dose of 100 mg/day to reach a peripheral white blood cell count of 4000–6000/μl.

Endomyocardial biopsy

Diagnostic endomyocardial biopsies were obtained from all patients as part of the routine postoperative management. Biopsies were performed more frequently during the early post-transplant period, when rejection is most likely to occur: weekly for the 1st month, every 2 weeks for the 2nd month, and then monthly. Additional endomyocardial biopsies were done any time rejection was suspected.

Statistical analysis

All values are expressed as mean ± SD. Statistical analysis was performed using Student's t-test. P values less than 0.05 were considered statistically significant.

Table 1 Comparison of the IL-1ra serum levels obtained in patients in the heart transplant (Htx) group and the control group. Data are given as mean ± SD. * $P < 0.01$ compared to the baseline values (Student's t-test)

Time	Control group ($n = 7$)	Htx group ($n = 12$)	P value
Baseline	507 ± 165	413 ± 180	NS
12 h	3980 ± 452*	4117 ± 459*	NS
Day 1	1739 ± 755*	1948 ± 767*	NS
Day 2	1383 ± 709*	1185 ± 833*	NS
Day 3	1176 ± 702	847 ± 537	NS
Day 4	1119 ± 527	998 ± 610*	NS
Day 5	1238 ± 701	1582 ± 1110*	NS
Day 6	850 ± 267	852 ± 488	NS
Day 7	785 ± 264	996 ± 801	NS
Day 8	729 ± 255	969 ± 1008	NS
Day 9	762 ± 327	935 ± 968	NS
Day 10	698 ± 267	1187 ± 1149	NS
Day 11	676 ± 284	905 ± 739	NS
Day 12	602 ± 258	806 ± 745	NS
Day 13	564 ± 235	715 ± 510	NS
Day 14	540 ± 263	681 ± 546	NS

Results

Study population

The mean age of the 12 patients undergoing orthotopic heart transplantation due to end-stage heart failure was 56 ± 3 years. The mean age of the control group was 53 ± 6 years (P = NS).

In the control group

Compared to baseline, the IL-1ra increased significantly 12 h after decrossclamping of the aorta (507 ± 165 vs 3980 ± 452 pg/ml, $P < 0.01$). After another 12 h, the serum levels decreased but still remained significantly elevated compared to the baseline values for another 2 days. From day 6 onwards, the serum levels were comparable to the baseline values (Table 1).

In patients undergoing heart transplantation

The pattern of IL-1ra serum levels was very similar to that in the control group. The highest level (4117 ± 459 pg/ml) was reached 12 h after starting the reperfusion of the allograft. Compared to baseline values (413 ± 180 pg/ml), the serum levels were significantly higher until 5 days after transplantation ($P < 0.01$). From day 6 until day 14, they remained fairly constant.

To provide more detailed information on the kinetics of IL-1ra serum levels, Table 2 shows the raw data obtained in the 12 patients after allograft transplantation. Comparison of the control group with the transplant group revealed no significant differences in IL-1ra serum levels throughout the entire observation period.

Endomyocardial biopsies in transplant recipients

During the observation period of 14 days, two endomyocardial biopsies were obtained from each patient. According to the standard criteria of the International Society for Heart and Lung Transplantation, an acute rejection was diagnosed in two patients on days 8 and 14, respectively (Table 3). Both patients were treated for acute rejection with three times 1000 mg methylprednisolone. The results of the endomyocardial biopsies of all patients are given in Table 3.

Table 2 IL-1ra serum levels of the patients after orthotopic heart transplantation. Patients S.A. and S.K.-H. showed a renewed and more pronounced elevation in serum levels for 2 days. This was accompanied by an acute allograft rejection

Time	S.A.	H.H.	N.W.	S.K.-H.	H.F.	L.S.	E.H.-W.	L.A.	M.M.	E.E.	Sch.A.	S.W.
Baseline	808	711	250	467	657	512	312	379	298	243	200	130
12 h	3849	3549	4096	3467	5574	3195	3999	4341	4352	3907	4493	4577
Day 1	1863	3395	3846	1618	1615	2712	614	1537	152	1481	902	2443
Day 2	1925	2536	1330	1197	612	3047	280	1107	247	419	290	1228
Day 3	1944	401	664	351	2042	421	1399	1229	326	776	318	302
Day 4	2375	368	642	301	2124	607	1299	1279	623	1576	562	228
Day 5	2455	3725	590	172	3519	894	1145	751	1371	3290	372	705
Day 6	2737	296	512	826	1615	789	897	1093	657	140	358	311
Day 7	4011	648	493	1193	2593	656	547	498	622	44	359	298
Day 8	4112	208	824	3877	901	394	65	458	263	16	277	234
Day 9	3456	188	745	4226	481	496	73	803	287	17	250	205
Day 10	2564	147	616	4116	564	889	82	3781	780	262	248	199
Day 11	1563	223	1319	3386	511	881	419	1789	63	211	250	249
Day 12	430	190	654	2889	444	795	346	143	111	3197	263	216
Day 13	1563	186	914	2569	404	745	139	848	121	432	245	416
Day 14	2760	203	470	1879	511	659	493	78	490	288	179	167

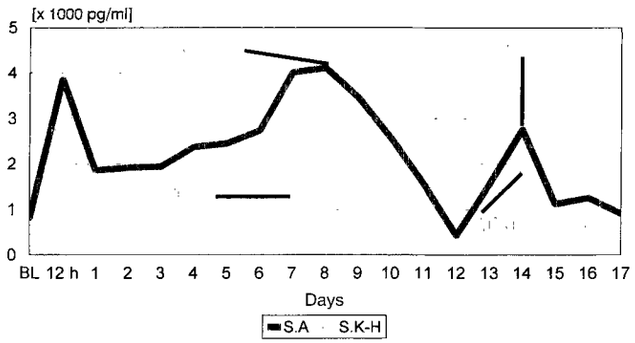

Fig. 1 The time courses of the IL-1ra serum levels in the two patients with allograft rejection. The second elevation after the initial rise was accompanied by an acute allograft rejection

Table 3 The results of endomyocardial biopsies (EMB) taken during the first 2 weeks after heart transplantation

Endomyocardial biopsy				
Patient ID	1st EMB	Treatment	2nd EMB	Treatment
S.A.	III.b.	Yes	I.a.	No
H.H.	I.a.	No	I.a.	No
N.W.	0	No	0	No
S.K.-H.	I.a.	No	III.a.	Yes
H.F.	0	No	0	No
L.S.	0	No	0	No
E.H.-W.	0	No	0	No
L.A.	0	No	I.a.	No
M.M.	0	No	0	No
E.E.	I.a.	No	0	No
Sch.A.	0	No	0	No
S.W.	0	No	I.a.	No

Correlation of IL-1ra serum levels and histological indices of rejection

Two of the twelve patients showed evidence of early allograft rejection. Patient S.A. presented diffuse lymphocytic infiltration with myocyte necrosis (Billingham IIIb.) on day 8 after transplantation. IL-1ra serum levels increased starting on day 4 and remained high for 3 days, i.e., from day 7 to day 9. When the endomyocardial biopsy was performed on day 8 and the diagnosis of acute allograft rejection was made, serum IL-1ra was at its highest level. Methylprednisolone pulse therapy resulted in a rapid decrease in serum IL-1ra to baseline values. A second endomyocardial biopsy obtained on day 14 was characterized by focal lymphocytic infiltrates (Billingham Ia.), indicating successful rejection therapy.

The endomyocardial biopsy of patient S.K.-H. showed multifocal lymphocytic infiltrates on day 14, consistent with acute allograft rejection (Billingham IIIa.). The kinetics of the IL-1ra serum levels were very similar to those of patient S.A. Starting on day 7, when the first endomyocardial biopsy showed only a focal lymphocytic infiltrate (Billingham Ia.), serum IL-1ra increased; it reached a maximum of 4226 pg/ml on day 9. The levels remained high for 4 days, until day 11. Without therapeutic intervention the serum levels decreased until day 14, when the diagnosis of acute allograft rejection was made (Table 2). Three additional blood samples were assayed during rejection therapy. Following pulse therapy with methylprednisolone, the IL-1ra serum level was comparable to the baseline value obtained prior to transplantation (Fig. 1).

To summarize, both patients showed (1) increasing IL-1ra serum levels prior to the diagnosis of acute allograft rejection by endomyocardial biopsy and (2) an elevated serum level above 4000 pg/ml for 2 days. The association of serum IL-1ra and graft rejection in the two patients is depicted in Fig. 1.

Discussion

Cytokines participate in the regulation and recruitment of cells in a variety of biological responses including inflammation and immune response due to allograft transplantation. IL-1ra is a naturally occurring inhibitor that is able to attenuate most of the known actions of the cytokine IL-1 [7], and its production by peripheral blood mononuclear cells is differentially regulated [1, 17]. Therefore, investigators have approached the determination of IL-1ra serum levels in different pathophysiological settings. For example, it has been shown that the production of IL-1ra is stimulated by experimental endotoxemia, indicating that IL-1ra is involved in the systemic inflammatory response [12]. The determination of IL-1ra in patients with extended thermal injury allows one to recognize patients who suffer predominantly from inhalative injuries. In this group of patients, IL-1ra appears to be a more sensitive marker of human inflammation than IL-1β or TNF [16]. Different surgical approaches and trauma cause changes in the production of different cytokines as well. Decker et al. showed that minimally invasive surgical procedures like laparoscopic cholecystectomies cause a reduced production of IL-1ra, and the lower serum levels were associated with a lesser activation of the immune system due to this elective operative procedure [5].

Since the late 1980s it is well known that the production if IL-1 is induced in patients undergoing cardiopulmonary bypass, and elevated levels of IL-1 have been associated with adverse clinical events following open heart surgery [13]. Nevertheless, the determination of its naturally occurring antagonist has not yet been performed. The results obtained from patients in our control group clearly indicate that cardiopulmonary bypass

per se activates the secretion of IL-1ra and that this increases within 12 h after decrossclamping of the aorta. Since, according to our study protocol, we only enrolled patients with an uneventful postoperative course, it seems that this initially elevated serum level may not possess predictive value for adverse events following open heart surgery.

Like many other immunosuppressive agents [3, 8, 14, 19], steroids are able to decrease cytokine secretion; glucocorticoids, in particular, have potent inhibitory effects on IL-1 production in tissue [15]. Sousa et al. showed that the inhalative application of glucocorticoids results in a significant decrease in epithelial IL-1β expression without altering the IL-1ra level [20], indicating immunosuppressive properties of IL-1ra that were proposed by data obtained from experimental studies [18].

Our initial immunosuppressive regimen consisted of the systemic administration of 1000 mg of methylprednisolone prior to the induction of anesthesia and just prior to reperfusion of the transplanted allograft. Similar patterns of IL-1ra serum levels in control patients and in patients undergoing orthotopic heart transplantation indicate that the production of IL-1ra was not suppressed by the administration of methylprednisolone as an immunosuppressive agent.

Since noninvasive techniques have been proposed as useful tools in the diagnosis of allograft rejection [11, 21], we correlated IL-1ra serum levels with the diagnosis of rejection as determined by endomyocardial biopsies. Two patients had an acute allograft rejection early after heart transplantation. These rejection episodes were characterized by a renewed, and much more distinct, increase in serum levels above 4000 pg/ml for at least 2 days. For plasma cytokine monitoring to be meaningful, it is important to define cut-off values of cytokine levels. As observed by Daniel et al., a cut-off value of 4000 pg/ml may be considered reliable for assessing a rejection episode and may differentiate rejection from infection in patients after renal transplantation [4]. In light of the fact that early graft rejection results in poor outcome of graft function, our data gave promising results that the determination of IL-1ra levels may predict a severe allograft rejection early after transplantation.

According to our preliminary results, IL-1ra is an early, but nonspecific, marker of immune activation in response to cardiopulmonary bypass. In two cases the production of this marker was reactivated by acute allograft rejection. Further studies are mandatory to confirm the predictive value of this cytokine for the detection of allograft rejection.

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