Discoordinate Modulation of Natriuretic Peptides During Acute Cardiac Allograft Rejection in Humans

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Background—Increased circulating levels of the cardiac polypeptide hormones atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP) may be observed after orthotopic cardiac transplantation. Both the hypertrophic and inflammatory processes in the allograft may contribute to this increase, but no mechanistic explanation has been suggested for this observation.

Methods and Results—Plasma immunoreactive ANF and BNP determinations were performed in 10 consecutive transplant patients. These were correlated with degree of rejection as reflected by histopathological findings at serial endomyocardial biopsies. Three patients had associated hemodynamic measurements and blood samples 24 hours before and after transplantation. All rejection episodes that received treatment were accompanied by a marked increase in BNP plasma levels to $>400$ pg/mL. Steadily increasing BNP levels preceded overt rejection as assessed by histopathological criteria. The increase in plasma BNP was not always accompanied by an increase in ANF, which suggests the specific upregulation of BNP gene expression during acute rejection episodes. Treatment of the acute rejection episodes led to a substantial decrease of BNP plasma levels.

Conclusions—The significant selective increase in plasma BNP levels found in the present study has not been previously described. This finding provides a new insight into the mechanism of allograft rejection and the modulation of natriuretic peptide synthesis and release. Furthermore, although preliminary, the data suggest that BNP plasma levels could form the basis for a new, noninvasive screening test to predict acute cardiac allograft rejection. Because treatment with the antilymphocyte monoclonal antibody OKT3 (murine monoclonal antibody to the CD3 antigen of the human T-cell) decreased BNP plasma levels, cytokine production by T-cells may mediate the selective increase in circulating BNP.

Key Words: transplantation ■ natriuretic peptides ■ atrial natriuretic factor ■ myocarditis ■ pathology

Under certain pathophysiological conditions, synthesis and release of the cardiac natriuretic peptides (NP) atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP) are significantly augmented in both atrial and ventricular cardiocytes. Increased production of these hormones by the ventricle is a hallmark of cardiac hypertrophy, failure, and inflammation. The former increase reflects the reexpression of the cardiac fetal phenotype seen with chronic hemodynamic overload, but the basis for the increased production of NP in myocarditis has not yet been defined.

We reported that after cardiac transplantation, ANF plasma concentrations remain elevated, reaching levels comparable to those found before transplantation despite the normalization of filling pressures and the renin-angiotensin-aldosterone system. Elevated BNP plasma levels have also been found after cardiac transplantation. Because BNP is predominantly of ventricular origin, it would be expected that replacement of the failing ventricle would decrease BNP plasma levels, although the transplanted ventricle may have sustained various types of stress. To substantiate this view, we determined BNP and ANF plasma levels in patients undergoing cardiac transplantation. Simultaneous right heart catheterization and hormonal blood assays were performed preoperatively and postoperatively, 24 hours postoperatively, and at each endomyocardial biopsy.

Patients and Methods

Ten consecutive patients undergoing orthotopic cardiac transplantation were prospectively studied. Right heart catheterization and hormonal blood assays were performed simultaneously. A right ventricular endomyocardial biopsy and blood sampling were performed weekly for 2 to 4 weeks, then every 1 to 4 months for the first year postoperatively. Whenever possible, patient blood samples were obtained preoperatively and 24 hours postoperatively. The group consisted of 7 men and 3 women, with a mean age of 56.5 years (range, 39 to 65 years). Seven patients had ischemic heart disease, 2 had idiopathic dilated cardiomyopathy, and 1 had valvular heart disease. All patients had end-stage heart failure, and 1 also had...
angina. Follow-up ranged from 4 to 40 weeks after transplantation (mean, 17 ±3 weeks).

Immunosuppression was induced with intravenous antithymocyte globulin and SoluMedrol. Maintenance of immunosuppression consisted of triple therapy based on prednisone, cyclosporine, and azathioprine. Rejection episodes were treated with OKT3, SoluMedrol, and methotrexate.

Biopsy specimens were graded according to the International Society of Heart and Lung Transplantation Standardized Grading System, which is based on the degree and distribution of lymphocytic inflammatory infiltration and the presence of cardiocyte necrosis (grade 2 and greater).

A total of 4 to 6 specimens that were obtained from routine percutaneous transvenous right ventricular endomyocardial biopsies were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned to obtain 4-μm step-sections through the entire block. Neither the pathologist nor the treating physicians knew the NP plasma levels.

Plasma ANF and BNP levels were determined by radioimmunoassay, as previously described, using specific antisera against human ANF<sub>99–126</sub> (Peninsula Laboratories) and human BNP<sub>77–108</sub> (Advanced ChemTech).

All results are presented as mean ± standard error of the mean. Comparisons between groups were made using the unpaired Student’s t test. P<0.05 was considered significant.

## Results

During the study period, 77 biopsies and assays were performed, with 8 episodes of rejection requiring treatment in 4 patients. In the 6 patients in whom there was no rejection, 42 biopsies and assays were performed, and in the 4 patients with rejection, 35 biopsies and assays were performed.

The mean level of postoperative BNP in patients without rejection was 198 ±12 pg/mL; it was 544 ±116 pg/mL in those with rejection (P=0.014). The average peak level of postoperative BNP was 293 ±33 pg/mL (range, 191 to 382 pg/mL) in patients without rejection and 1198 ±720 pg/mL (range, 407 to 3315 pg/mL) in patients with rejection. In the group of patients without rejection, the mean level of postoperative plasma ANF was 230 ±15 pg/mL; in those with rejection, the mean level was 240 ±16 pg/mL. The average peak level of postoperative ANF was 342 ±48 pg/mL (range, 193 to 484 pg/mL) in those patients without rejection and 318 ±64 pg/mL (range, 183 to 525 pg/mL) in those patients exhibiting rejection. Overall, ANF plasma levels were slightly lower than BNP levels (239 ±12 pg/mL [n=84] versus 279 ±35 pg/mL [n=84]).

Figure 1 shows the hormonal profiles of 2 patients who did not experience significant rejection during the study period. In the first patient (Figure 1A), the range of postoperative BNP plasma levels was 84 to 236 pg/mL. In second patient (B), range of BNP plasma levels postoperatively was 187 to 383 pg/mL. Graphs illustrate that plasma ANF and BNP levels after transplantation follow unique patterns in individual patients. None of 6 patients without rejection had postoperative BNP plasma levels ≥400 pg/mL.

Figure 2 shows the hormonal profiles of 3 patients who had significant rejection. In Figure 2A, the patient had grade 3A rejection on the sixth biopsy, associated with a rise in BNP plasma level from 257 to 407 pg/mL. This patient was treated with SoluMedrol; rejection resolved and BNP plasma levels decreased to 271 pg/mL. The first rejection episode in the patient shown in Figure 2B (grade 3A rejection), detected at the second biopsy, was associated with a rise in BNP plasma levels from 688 to 1294 pg/mL. The patient was treated with SoluMedrol; however, the repeat biopsy 1 week later showed worsening of rejection (grade 4), with a further rise in the BNP plasma level to 3315 pg/mL. The patient was treated with OKT3 and underwent biopsy 1 week after therapy. This biopsy showed resolution of the rejection, and BNP plasma levels decreased to 842 pg/mL. The patient subsequently had 2 further episodes of rejection. On the seventh week after transplantation, BNP plasma levels again rose to 1234 pg/mL and the biopsy showed grade 2 rejection. On the eleventh week, the biopsy again was graded as having grade 2 rejection; however, on this occasion, there was no rise in BNP plasma levels. ANF plasma levels in this patient remained essentially constant throughout the sampling period. The patient in Figure 2C experienced 4 episodes of rejection. On the third week after transplantation, BNP levels rose from 628 to 890 pg/mL, and the biopsy was graded 3A. The patient received OKT3, and the biopsy 1 week later showed resolving rejection, with the BNP concentration falling to 288 pg/mL. At this time, the patient received intravenous steroids. One week later, however, the biopsy showed persistent rejection (grade 3A), with a rise in BNP to 1237 pg/mL. The patient received a further course of OKT3 and SoluMedrol. After the fourth rejection episode during week 9, the patient
was treated with methotrexate. In all of these patients, rejection episodes were associated with a BNP level of $400 \text{ pg/mL}$.

**Discussion**

### Natriuretic Peptides and Cardiac Allograft Rejection

In previous studies on ANF plasma levels after cardiac transplantation, we found that despite normalization of filling pressures after a transient period of elevation as well as normalization of the renin-angiotensin-aldosterone system, ANF remained elevated. Other investigations showed a lack of normalization of either ANF or BNP plasma levels after cardiac transplantation in humans. To date, no specific mechanism has been identified that would explain this phenomenon. We advanced the view that cyclosporine actions on endothelial cells leading to endothelin overproduction could explain, at least in part, the high levels of plasma ANF after cardiac transplantation. We further suggested that this overproduction of ANF may be key in ameliorating cyclosporine-induced hypertension brought about by increased vascular smooth-muscle sensitivity to pressor agents induced by cyclosporine-mediated changes in calcineurin gene expression.

A role for rejection as a determinant of high ANF plasma levels could also be suspected because rats with a rejecting heart had increased plasma ANF levels. However, the published human data does not support a relationship between inflammation and increased plasma ANF levels. Part of the increased expression of NP genes in chronic myocardial inflammation could be related to the hypertrophy that has been reported to occur as early as 1 week in transplanted hearts and that remains evident for several years afterward. The determinants of this hypertrophic response are not known; no correlation has been found between microscopic cardiocyte hypertrophy and cyclosporine treatment or ischemic time. In experimental acute Chagas myocarditis, we demonstrated that ANF plasma levels increase in the absence of cardiac failure or hypertrophy.

### Mechanism Underlying Increased NP Plasma Levels During Acute Rejection

Because the BNP plasma levels were highest in patients with rejection grades 3 and 4, which denote cell necrosis and mononuclear cell infiltration, it is important to consider nonspecific BNP leakage from cardiocytes as a possible source of increased BNP plasma levels during acute rejection episodes. From studies of BNP gene expression in myocardial infarction and myocarditis, however, it has been established that both of these processes are accompanied by increased BNP gene expression and not mere cell leakage. Furthermore, in the present studies, we found an increase in BNP plasma levels before the biopsy showing histopathological grades associated with cell necrosis. The mechanism underlying the increase in circulating BNP during acute rejection episodes observed in our studies may be based on the fact that treatment with OKT3 reverses this increase. The monoclonal antibody OKT3 prevents T-cell proliferation and function. This clearly relates increases in circulating BNP to T-cell activation and cytokines. This relationship, however, has not been addressed to date.

From the results obtained in the present investigation in those patients for whom plasma BNP levels were available preoperatively and postoperatively, it seems that in some cases, BNP tends to decrease after transplantation, suggesting that the recipient’s ventricle was at least partly the source of the increased levels of plasma BNP found preoperatively. The
fact that the further increase in plasma BNP observed in association with rejection episodes occurs with or without an accompanying increase in plasma ANF levels suggests that these hormones may be regulated in a discoordinate manner.

Our previous experimental work\(^2,3,30\) suggests that ANF plasma levels may be a measure of extracellular volume status whereas BNP levels reflect cardiac hypertrophy and ventricular dysfunction.\(^31,32\) These underlying events, superimposed to the specific effect(s) of the inflammatory process and together with varying degrees of the preservation of renal function in individual patients, may explain the variations in NP ratios that were found.

**Intracardiac Pressures and NP Production in Cardiac Allografts**

Three of the patients included in this study had right atrial pressure and pulmonary wedge pressure consistently determined at the time of blood sampling for NP determination. Statistical analysis showed that a significant linear correlation existed between right atrial pressure and pulmonary wedge pressure in these patients but not between these pressures and ANF or BNP plasma levels or between NP plasma levels themselves (data not shown). Lack of correlation between cardiac filling pressures and ANF plasma levels has been previously reported for transplant patients,\(^4\) although another study\(^13\) found a good correlation between these parameters. It is likely that the differences in volume status, cardiac factors, and degrees of rejection, as outlined in the above paragraph, may explain these differences. It is of interest that inspecting the correlation curves reveals that outliers in correlations between NPs or between NPs and cardiac filling pressures are often those NP values that are associated with clinically significant rejection episodes requiring treatment. These findings clearly suggest that in those cases, rejection, not filling pressures, is the major determinant of NP, particularly BNP plasma levels. The number of cases with cardiac pressure determinations, however, is too small to reach definitive conclusions at this time.

**BNP Plasma Levels as a Potential Screening Test for Acute Cardiac Allograft Rejection**

The data that we obtained is limited because of the small number of patients included; however, thus far they suggest that rejection episodes needing therapeutic intervention are associated with BNP plasma levels \(\geq 400\) pg/mL. The data presented here also suggest that pooling data on cardiac hormone levels from the general population of cardiac transplant patients could lead to nonsignificant associations between increasing plasma BNP and clinically significant rejection episodes because each patient evolves from his or her own baseline level of BNP.

It should be emphasized that in no instance did we find BNP plasma levels \(>400\) pg/mL without an association with a rejection episode. This finding suggests that the determination of plasma BNP should have few false positives when used as a screen for rejection. Rejection episodes that required additional immunosuppressive therapy were always associated with and preceded by an increase in BNP plasma levels. In 1 patient who had a difficult histopathological diagnosis and a tentative rejection grade of 3A (multifocal moderate rejection), which is usually associated with additional immunosuppressive therapy, BNP levels were in a downward trend; they measured 40 pg/mL on the day of the biopsy. Clinically, no additional treatment was given to this patient, despite the histopathological grade, and none was required 3 weeks later when the biopsy was graded 0 and BNP plasma levels were 25 pg/mL. This incident emphasizes the occasional difficulty encountered with biopsy interpretation and sampling and the potential value of investigating plasma BNP levels as a marker of rejection. Finally, in all patients studied, mild rejection (lymphocytic infiltrate without cardiocyte necrosis) leading to a more serious rejection episode was accompanied by an increase in plasma BNP levels. This suggests that BNP plasma levels may be useful in predicting the outcome of a mild rejection episode.

The data presented here suggest that a larger study, leading to the establishment of a sensitive, specific, and predictive screening procedure for cardiac allograft rejection based on the determination of BNP plasma levels, is warranted. Furthermore, the fact that BNP may be specifically upregulated during acute rejection episodes, likely through a process related to T-cell cytokine production, is worthy of further investigation given the potential value of this finding in understanding the regulation of NP gene expression and its role in myocardial inflammation.

**Acknowledgments**

We thank Michelle Stevenson, Amalia Ponce, Tara Hetherington, Christine Struthers, and Carole Frost for their excellent assistance. We also thank Dr Virginia Walley for her support of this project. Supported by grants from the Heart and Stroke Foundation of Ontario (T3038) and the Medical Research Council of Canada (MT13418).

**References**


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Circulation. 1999;100:287-291
doi: 10.1161/01.CIR.100.3.287

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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