EXHALED NITRIC OXIDE CONCENTRATION IN PATIENTS AFTER HEART TRANSPLANTATION

Nitric oxide (NO) is present in exhaled air in minimal concentration of several parts per billion (ppb) in healthy humans. In the present study we prospectively investigated how heart transplantation treated with oral immunosuppressive drugs based on ciclosporine A influences the exhaled NO concentration (exNO). The study was performed in 17 patients after heart transplantation in various time after procedure and 15 non-smoking healthy volunteers as a control group. Patients after heart transplantation were free of clinical signs of rejection. End-tidal concentration of exNO was measured by the use of a chemiluminescence method. We found no statistically significant differences in the exNO level between patients after heart transplantation and healthy controls (6.81 ±2.70 part per billion (ppb) in the transplant group vs. 6.01 ±3.43 ppb in the control group). We conclude that heart transplantation and immunosuppressive therapy do not influence the exhaled NO concentration.

Key words: exhaled nitric oxide, heart transplantation

INTRODUCTION

Nitric oxide (NO) is present in exhaled air in minimal concentration of several parts per billion (ppb) in healthy humans. Exhaled nitric oxide (exNO) is produced by the endothelial cells in the airways and alveoli and only a small portion is produced by pulmonary vessels in which neuronal NO synthase is expressed (1, 2, 3). It has been found that patients with chronic heart failure have a lower level of exNO in comparison with healthy volunteers (4, 5), although in other heart conditions, e.g., in chronic coronary artery disease exNO may be in a
normal range (6). There is little data concerning exNO levels in heart transplant patients. Nor do we know how immunosuppressive drugs, such as like a cyclosporine A, affect the level of exNO. Patients after lung transplantation have elevated nitric oxide concentrations in exhaled air during acute rejection (7), which has also been observed in animal models (8).

In the present study we set out to examine exNO concentrations in heart transplant patients and compared it with that in healthy control subjects.

SUBJECTS AND METHODS

Study protocol was approved by a local Ethics Committee (approval No NN-013-136/I/00 from the 2000.09.14) and informed consent was obtained from all participants.

exNO was examined in 32 male participants. There were 17 male patients after heart transplantation at various time after the procedure. The patients were free from clinical signs of rejection. All of them took immunosuppressive therapy. The following immunosuppressant drugs were employed: cyclosporine A in a mean dose of 217.3 mg/day to maintain serum levels between 200-300 ng/ml, azathioprine (Immuuran; 50-100 mg/day; in 7 patients), and the steroid prednisone (Encorton; 20-70 mg/day; in 10 patients). Adjunctive therapy consisted of calcium channel blocking drugs, ACE inhibitors, and statins in 41% of the patients, and beta blockers in 12% of the patients. A control group consisted of 15 non-smoking healthy male volunteers. These subjects did not use any medications.

The exclusion criteria included: age below 18, any signs of infection, chronic lung disease, the use of inhalational medications, and smoking habit. Ex-smokers were classified as non-smokers if they had stopped smoking one or more years before the study took place. Smoking cessation at a later date was considered as a reason for exclusion from the study.

exNO was assessed using a high resolution chemiluminescence analyzer (280 NOA, Sievers, Boulder, Colorado, USA) adapted for on-line recording of NO concentration. An internal restrictor in the breathing circuit allowed expiration against a resistance of 10 cmH\text{2}O to keep the soft palate closed and to prevent contamination of the exhaled air with nasal NO. A single breath measurement was performed at rest at a constant flow of 200 ml/min. Plateau values were obtained from the exNO single breath curve. The mean value from six consecutive and reproducible measurements was automatically calculated and considered for analysis. The whole procedure of the exNO measurement was performed according to the recommendations of the European Respiratory Society (9).

All data are shown as means ±SD. The data distribution was analyzed using the Kolmogorow-Smirnow test. Depending on the result of this analysis, they were further analyzed with a t-test for repeated measures or Wilcoxon test. Differences between groups were assessed with a t-test for independent samples or Mann-Whitney test. Differences in frequency distribution were analyzed with the Fischer exact test. P<0.05 was considered significant.

RESULTS

Patients after heart transplantation were compared with healthy volunteers. Weight and body mass index were similar in both groups. There were some differences in the demographic data between both groups. Patients after heart transplantation were older and shorter, exNO concentrations were similar in both groups (Table 1).
DISCUSSION

There is lack of data concerning exNO levels in heart transplant recipients. We investigated 17 patients after heart transplantation in a good physical condition and without rejection. They were on immunosuppressive therapy with cyclosporine A as a main drug. In our study we were not able to show any significant differences in exhaled nitric oxide concentrations between patients with heart transplantation and a group of healthy volunteers. Zegdi et al (10) compared seven patients after heart transplantation with eight lung transplant recipients and did not find any differences in exhaled nitric oxide levels. Those patients also were free of any signs of infection or rejection. There are also other studies that demonstrate that exNO concentration in lung transplant patients, with no signs of rejection or bronchiolitis obliterans, is similar to that in the healthy population (11).

In the present study heart transplant patients were significantly older than healthy volunteers. We had a difficulty in finding age-matched healthy participants who would not take any medications. Therefore, the exact age-matched comparison could not be achieved. Nevertheless, in one of our previous studies we found lack of association between age and exNO levels (12). Ekroos et al (13) have not found any correlations between age and exNO levels in their group of 26 patients either. Similar findings also are mentioned by Kharitonov and Barnes (14) in their review article.

These results could be affected by the signs of rejection. The level of exNO increases to high values in the presence of acute rejection in lung transplant subjects in animal and human studies (7, 8), although Fisher et al (7) have found that there is no change in the exNO concentration during vascular rejection in lung transplantation. This suggests that the exNO assessment could be useful in the diagnosis of acute rejection in lung transplant patients (15). We do not know if that could be extrapolated to heart transplant patients and further investigations are necessary to this end. In our study population of 17 patients after heart transplantation none have a rejection grade higher than 1B at the time of the investigation.

Table 1. Demographic data and exNO concentrations

<table>
<thead>
<tr>
<th>Parametr</th>
<th>Group I (n=17)</th>
<th>Group II (n=15)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.1 ±7.6</td>
<td>34.8 ±10.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171 ±6.4</td>
<td>177 ±8.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.0 ±9.2</td>
<td>80.1 ±11.8</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (body mass index)</td>
<td>26.3 ±3.0</td>
<td>25.5 ±3.4</td>
<td>NS</td>
</tr>
<tr>
<td>exNO concentration (ppb)</td>
<td>6.81 ±2.70</td>
<td>6.01 ±3.43</td>
<td>NS</td>
</tr>
</tbody>
</table>

Group I -heart transplant patients; Group II -control subjects
The patients of the present study were on immunosuppressive therapy based on cyclosporine A, corticosteroids, and azathioprine after heart transplantation. It is difficult to say how these drugs affect the exNO level in humans, since the relevant literature is scant. Attur et al (16) have investigated the influence of cyclosporine on NO production in a murine macrophage colony. The authors have shown a diminished activity of inducible nitric oxide synthase (iNOS) in the presence of cyclosporine. Mora et al (8) have performed an investigation on rats. After lung transplantation, the authors have started immunotherapy with cyclosporine in one group and a second group of rats was left without any medication. The exNO level was compared between the groups and it was found to be remarkably high in the untreated group in which the rats had signs of acute rejection. The group of rats after lung transplant treated with cyclosporine, in which there were no signs of rejection, had a low exNO level, which was comparable with that in the non-transplanted control animals. However, these results could hardly be extrapolated to humans.

The effects of corticosteroid drugs on exNO are well documented. The inhalant forms of these drugs decrease exNO in patients with asthma, due likely to a reduction in local inflammation (16). Corticosteroids given orally exert a weaker effect on exNO. Yates et al (18) have not found any differences in the level of exNO after oral corticosteroids in healthy volunteers.

In conclusion, exhaled NO concentration is not affected by heart transplantation in patients on immunosuppressive therapy based on cyclosporine.

**Acknowledgments:** This publication was supported by grant from the State Committee for Scientific Research no. 3PO5B06025.

**REFERENCES**


Author’s address: Paweł Nadziakiewicz, Department of Cardiac Anesthesia, Silesian Center for Heart Diseases, Szpitalna 2 St., 41-800 Zabrze, Poland; e-mail: nadzial@poczta.onet.pl