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Neuroendocrine changes during the evolution of doxorubicin-induced left ventricular dysfunction in adult lymphoma patients

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ABSTRACT

Doxorubicin-induced cardiotoxicity was used as a model to prospectively investigate neuroendocrine changes during the development of left ventricular dysfunction. Radionuclide ventriculography, frequency domain analysis of heart rate variability (HRV), and plasma noradrenaline and natriuretic peptide measurements were performed in 27 adult lymphoma patients at baseline and after cumulative doxorubicin doses of 200, 400 and 500 mg/m². The left ventricular ejection fraction (LVEF) decreased from 58.1 ± 1.4% to 50.3 ± 1.1% (P < 0.001) and 49.3 ± 1.7% (P < 0.001) after cumulative doxorubicin doses of 400 and 500 mg/m² respectively. With a doxorubicin dose of up to 400 mg/m² there was an increase in sympathetic tone, characterized by a decrease in the normalized high-frequency (HFnu) power (P < 0.011), and increases in the normalized low-frequency (LFnu) power (P < 0.011), the LF/HF ratio (P < 0.021) and the plasma noradrenaline concentration (P < 0.034). The decrease in LVEF was correlated with the changes in LFnu and HFnu power (r = 0.540, P = 0.012) and LF/HF ratio (r = -0.452, P = 0.04). However, after the cumulative doxorubicin dose of 500 mg/m² the changes in HRV components and plasma noradrenaline levels returned towards baseline. This was accompanied by increased concentrations of plasma atrial natriuretic peptide (P < 0.004) and brain natriuretic peptide (P < 0.021). Our findings suggest that doxorubicin-induced left ventricular dysfunction is associated with an early change in sympathovagal balance towards sympathetic predominance. Along with further progression of left ventricular dysfunction, there is an attenuation of sympathetic tone, which may be attributable to sympahto-adrenal inhibition by increased secretion of natriuretic peptides.

INTRODUCTION

Cumulative, dose-related cardiomyopathy is a well known side effect of doxorubicin therapy. Histological findings in anthracycline-induced cardiomyopathy include myofibrillar loss, myocyte vacuolization, and eventually death of the myocyte [1]. The incidence of congestive heart failure during doxorubicin therapy has been reported to be approx. 3% at a dose of 400 mg/m² and 7% at a dose of 500 mg/m² [2], but an asymptomatic decline in the left ventricular ejection fraction (LVEF) is far more common [3]. Congestive heart failure is associated with neuroendocrine activation, manifested by increases in

Key words: autonomic nervous system, doxorubicin, heart rate variability, left ventricular dysfunction, natriuretic peptides.
Abbreviations: ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CHOP, cyclophosphamide, doxorubicin, vincristine and prednisolone; HF, high-frequency; HFnu, normalized high-frequency; HRV, heart rate variability; LF, low-frequency; LFnu, normalized low-frequency; LVEF, left ventricular ejection fraction; MSNA, muscle sympathetic nerve activity.
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sympathetic activity and plasma concentrations of noradrenaline, activation of the renin–angiotensin–aldosterone system and the release of vasoactive substances such as natriuretic peptides [4]. Sympathetic activation, as assessed by increased muscle sympathetic nerve activity (MSNA) [5] or increased plasma noradrenaline concentrations [6], has also been demonstrated in patients with mild clinical congestive heart failure. Decreased heart rate variability (HRV) and changes in the frequency components of HRV suggesting increased sympathetic tone have been reported in congestive heart failure [7–9]. However, these studies are cross-sectional, and to our knowledge no prospective studies have investigated the temporal changes in HRV during the evolution of left ventricular dysfunction. Even less is known about neuroendocrine activation during anthracycline treatment.

The aim of the present study was to investigate prospectively the changes in cardiac autonomic control and their association with natriuretic peptides during the development of left ventricular dysfunction in lymphoma patients receiving doxorubicin-based chemotherapy.

METHODS

Patients
A total of 30 consecutive adult patients (≤75 years of age) with previously untreated non-Hodgkin’s lymphoma scheduled to receive CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone) chemotherapy were enrolled in the study. The patients were regarded as eligible for study entry if they had not received prior anthracycline therapy or mediastinal irradiation. A history of heart failure was also considered as an exclusion criterion. Two patients died early during the treatment due to progressive lymphoma, and one patient was excluded from the analysis because of multiple ectopic beats at baseline. Thus the study population consisted of 27 patients (10 women and 17 men), with a mean age of 53 years (range 22–75 years). Six patients (22%) had a pre-existing cardiovascular disease (four patients had WHO class I–II hypertension, one patient had suffered from a prior myocardial infarction and one patient experienced paroxysmal attacks of atrial fibrillation). Five patients received β-adrenoceptor blocking drugs, and two patients were on diuretics at study entry. No changes were made in the patients’ medication during the study period.

Approval for the study was obtained from the local ethical committee, and the patients provided written informed consent before the study.

Chemotherapy
The CHOP chemotherapy was administered in standard doses. Cyclophosphamide 750 mg/m², doxorubicin 50 mg/m² and vincristine 1.4 mg/m² (maximum 2 mg) were given intravenously on day 1, and prednisolone 100 mg was given orally on days 1–5. Doxorubicin was administered as a 30 min infusion. The cycle was repeated every 3 weeks. At least two additional cycles of CHOP were given after complete remission (maximum 10 cycles). Doxorubicin was discontinued if LVEF decreased below 45%. No radiotherapy was given during the study period.

Study protocol
Cardiac systolic function and cardiac autonomic regulation at rest were assessed at baseline and after cumulative doxorubicin doses of 200, 400 and 500 mg/m². In addition, plasma concentrations of noradrenaline, atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) were measured at the same time points.

ECG and blood pressure recordings
For the assessment of cardiac autonomic regulation, a surface ECG and a continuous non-invasive arterial blood pressure signal from the left middle finger (Finapres; Ohmeda, Englewood, CO, U.S.A.) were recorded with a computerized data acquisition and analysis package (CAFTS software; Medikro Ltd, Kuopio, Finland) [10]. The signals were digitized with a temporal resolution of 200 Hz/channel and an amplitude resolution of 12 bits. The digitized signals were stored on an IBM-compatible microcomputer for subsequent analysis.

Cardiac autonomic recordings at rest
The patients were studied during 10 min of controlled breathing (following a metronome at 0.2 Hz) in the supine position. A 5-min stationary ECG recording, free of ectopic beats, in the middle of the resting period was used for the assessment of cardiac autonomic control at rest.

Assessment of cardiac autonomic regulation
Cardiac autonomic regulation during the different stages of the study were estimated from the ECG recordings using frequency domain analysis of HRV. After detrending (first degree) a modified covariance autoregressive model (fixed model order 14) was used to obtain power spectral estimates of HRV. The power spectrum was quantified as total power (frequency range from 0 to 0.75 Hz), low-frequency (LF) power (range 0.04–0.15 Hz) and high-frequency (HF) power (range 0.15–0.40 Hz). Signal powers of each band were calculated as integrals under the respective power spectral density function and expressed in absolute units (ms²). LF and HF power were also measured in normalized units \[LF_{\text{nu}} = (LF/LF + HF); \text{HF}_{\text{nu}} = (HF/LF + HF)\]. In addition, the LF/HF ratio was calculated [11].
Radionuclide ventriculography

LVEF was assessed by radionuclide ventriculography using standard techniques [12,13]. Left ventricular dysfunction indicative of doxorubicin-induced cardiotoxicity was defined as a decrease in LVEF of more than 10% to a final LVEF of < 50% [14].

Neurohumoral variables

Blood samples from the antebrachial vein were withdrawn into chilled tubes containing 1.5 mg of K$_2$EDTA/ml of blood after the patient had been in a supine position for 30 min (at 08.00 hours). The whole blood was centrifuged, and plasma was frozen immediately and

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Figure 1  Changes in HRV and LVEF during doxorubicin therapy

The x-axis shows the cumulative doxorubicin dose in mg/m$^2$. Values are means ± S.E.M. Significance of differences: * $P < 0.05$, ‡$P < 0.001$ compared with baseline.
stored at \(-70^\circ\text{C}\) until used. Plasma noradrenaline concentrations were analysed by HPLC combined with electrochemical detection [15]. ANP and BNP were extracted from plasma using Sep-Pak C\(_{18}\) cartridges [16] and were analysed by RIA as described previously [17].

**Statistical methods**

All calculations were performed with the SPSS/PC statistical program (version 7.5.1; SPSS Inc., Chicago, IL, U.S.A.). The frequency components of HRV were analysed after logarithmic transformation. The differences for continuous variables were compared with multivariate analysis of variance (MANOVA). Paired, two-tailed Student’s \(t\) tests were applied for post hoc analyses. The correlation between variables was studied using Spearman’s correlation test. Additional subgroup analyses for patients with and without left ventricular dysfunction, as defined on the basis of a decrease in LVEF, were performed using the Mann–Whitney \(U\) test for continuous variables and with the chi-square test for nominal data. A \(P\) value of \(<0.05\) was considered as statistically significant. The data are expressed as means ± S.E.M. unless indicated otherwise.

**RESULTS**

From the original patient population, 27 patients were evaluable for analyses. Of these, 23 patients received 10 cycles and four patients received eight cycles of CHOP (cumulative doxorubicin doses of 500 mg/m\(^2\) and 400 mg/m\(^2\) respectively). Clinical congestive heart failure developed in two patients (7\%) at 1 month and 10 months after the last dose of doxorubicin (cumulative doxorubicin dose of 500 mg/m\(^2\)).

**Left ventricular systolic function**

LVEF decreased from 58.1 ± 1.4\% to 52.3 ± 1.1\% \((P < 0.001)\), 50.3 ± 1.1\% \((P < 0.001)\) and 49.3 ± 1.7\% \((P < 0.001)\) after cumulative doxorubicin doses of 200, 400 and 500 mg/m\(^2\) respectively (Figure 1).

**Cardiac autonomic regulation at rest**

No statistically significant changes were found in absolute total power, HF power or LF power after eight courses of CHOP (cumulative doxorubicin doses of 400 mg/m\(^2\)). However, there was a significant decrease in HF nm (from 0.54 ± 0.04 to 0.43 ± 0.04; \(P = 0.011\)) and increases in LF nm (from 0.46 ± 0.04 to 0.57 ± 0.04; \(P = 0.011\)) and the LF/HF ratio (from 1.25 ± 0.29 to 2.15 ± 0.44; \(P = 0.021\)) (Figure 1). Significant correlations were observed between the decrease in LVEF and the changes in LF nm and HF nm \((r = 0.540, P = 0.012)\) and the increase in the LF/HF ratio \((r = -0.452, P = 0.04)\). In multivariate analysis the changes in LF nm and HF nm or the LF/HF ratio were not associated with age \((P = 0.279\) and \(P = 0.280\) respectively) or with the cumulative dose of vincristine \((P = 0.624\) and \(P = 0.618\) respectively). Interestingly, after 10 courses of CHOP (cumulative doxorubicin dose of 500 mg/m\(^2\)), the changes in HRV moved back towards the baseline values (Figure 1).

**Neurohumoral variables**

The plasma noradrenaline concentration was 1.1 ± 0.1 nmol/l at baseline. It had increased to 1.4 ± 0.2 nmol/l after eight courses of CHOP \((P = 0.034)\), and decreased again to 1.3 ± 0.2 nmol/l \((P = 0.144)\) after 10 courses of CHOP (Figure 2). The concentrations of plasma ANP and BNP were 16.8 ± 1.3 pmol/l and 3.4 ± 0.4 pmol/l.
Neuroendocrine changes in left ventricular dysfunction

Figure 3 Changes in HRV and LVEF during doxorubicin therapy in patients with (right curves; n = 10) and without (left curves; n = 17) left ventricular dysfunction

The x-axis shows the cumulative doxorubicin dose in mg/m². I, no LV dysfunction; —, with LV dysfunction. Values are means ± S.E.M. Significance of differences: *P < 0.05, †P < 0.01, ‡P < 0.001 compared with baseline.

respectively at baseline. They remained unchanged after four courses of CHOP (cumulative doxorubicin dose of 200 mg/m²). After this, plasma ANP increased to 23.0 ± 2.5 pmol/l after 10 courses of CHOP (P = 0.004), and plasma BNP to 5.4 ± 0.8 pmol/l (P = 0.013) and 8.8 ± 2.1 pmol/l (P = 0.021) after eight and 10 courses of CHOP respectively (Figure 2).

Cardiac autonomic changes in patients with and without left ventricular dysfunction

Ten patients (37%) developed a decrease in LVEF of > 10% to a final LVEF of < 50%, which was our definition for subclinical left ventricular dysfunction. There were no significant differences at baseline in age, gender, hypertension, the use of β-adrenoceptor blocking drugs, the cumulative dose of vincristine, LVEF or HRV variables between the patients with and without subclinical ventricular dysfunction. The final LVEF in patients with subclinical left ventricular dysfunction was significantly lower than in patients without subclinical heart failure (41.7 ± 2.0% and 53.4 ± 1.6% respectively; P < 0.001) (Figure 3). After a cumulative doxorubicin dose of 400 mg/m², HFnu had decreased from 0.60 ± 0.07 to 0.38 ± 0.06 (P = 0.001), LFnu had increased from 0.40 ± 0.07 to 0.62 ± 0.06 (P = 0.001) and the LF/HF ratio had increased from 0.81 ± 0.25 to 2.34 ± 0.62 (P = 0.006) in patients with left ventricular dysfunction, whereas the changes in HRV remained non-significant in patients without a fall in LVEF (Figure 3). In addition, the changes in HFnu and LFnu from baseline were greater (P = 0.016) in patients with left ventricular dysfunction than in those without.

DISCUSSION

Our study design provided us with a unique opportunity to prospectively investigate neurohumoral changes during the evolution of left ventricular dysfunction in humans. The novel finding of this study is that doxorubicin-induced subclinical left ventricular dysfunction is associated with a very early change in cardiac autonomic regulation, characterized by a change in sympathovagal balance towards sympathetic predomi-
nance. Interestingly enough, however, this early sympathetic activation was attenuated on the continuation of treatment. Furthermore, the decrease in sympathetic tone was associated with increased secretion of natriuretic peptides, and may be attributable to their sympatho-inhibitory effects. Although our study investigated the effects of anthracycline therapy on cardiovascular neurohumoral regulation, the results may have more general applications to the development of heart failure of any origin.

Sympathetic activation, as assessed by increased plasma noradrenaline concentration [18], increased noradrenaline spillover into plasma [19] and increased MSNA [20], has been demonstrated in patients with severe congestive heart failure and also in patients with less severe left ventricular dysfunction [5,6]. Cardiovascular autonomic control can also be assessed by frequency domain analysis of HRV. Cardiac vagal activity is considered to be the major contributor to the HF component, whereas the LF component, and particularly the LF/HF ratio, are thought to reflect sympathovagal balance or sympathetic modulation of cardiac autonomic control [11]. Reduced HRV and changes in the spectral components of HRV suggesting increased sympathetic tone have been reported in patients with heart failure [7–9,21]. However, the alterations in cardiac autonomic control during the evolution of left ventricular dysfunction have been evaluated prospectively only in animal models.

We found significant increases in \( LF_{nu} \) and the LF/HF ratio and a significant decrease in HF\(_{nu} \) after the cumulative doxorubicin dose of 400 mg/m\(^2\), indicating a shift of cardiac autonomic regulation towards sympathetic predominance. This was supported by a simultaneous increase in the plasma noradrenaline concentration. In addition, the changes in \( LF_{nu} \), HF\(_{nu} \) and LF/HF ratio were correlated with the decrease in LVEF and were most evident in patients with left ventricular dysfunction, suggesting a compensatory response to impaired left ventricular function. However, after the cumulative doxorubicin dose of 500 mg/m\(^2\), \( LF_{nu} \), the LF/HF ratio and the plasma noradrenaline concentration decreased, concomitant with an increase in HF\(_{nu} \). This was accompanied by increases in plasma ANP and BNP concentrations. We hypothesize that the attenuation of the sympathetic tone may be attributable to the increased secretion of natriuretic peptides. This is in line with previous observations whereby ANP infusion in normal subjects has been shown to inhibit sympathetic outflow, characterized by decreases in MSNA [22] and the LF/HF ratio [23]. In addition, ANP infusion has been demonstrated to inhibit adrenaline and noradrenaline release in dogs [24].

Our results are in accordance with cross-sectional studies in which more advanced left ventricular dysfunction has been shown to be characterized by a decreased LF component of HRV and a decreased LF/HF ratio [21,25,26]. The decrease in LF power during the development of left ventricular dysfunction may be attributed in part to down-regulation of cardiac \( \beta_1 \)-adrenergic receptors, leading to reduced responsiveness of the sino-atrial node to high levels of sympathetic activation [27].

An increase in the LF component of HRV in response to doxorubicin-induced heart failure has been documented in an animal model [28]. Effects of anthracycline therapy on HRV in humans have been reported previously [29–33]. Three of these studies were retrospective [29,31,32], and one study evaluated cardiac parasympathetic control [30]. In the study by Postma et al. [31] an increase in LF components and a decrease in HF components of HRV were observed in children treated with higher cumulative doxorubicin doses exceeding 400 mg/m\(^2\). The patients were evaluated a median of 8.9 years after the treatment with doxorubicin. Tjeerdema et al. [32] reported a decrease in total power, LF power and HF power of HRV in women treated with epirubicin, doxorubicin or mitoxantrone for breast cancer. The patients were studied at a mean of 29 months after the end of chemotherapy and the results were compared with those from age-matched controls. However, in the only prospective study published so far, no changes in HRV were observed in patients treated with relatively low doses of epirubicin for breast cancer [33].

There are some limitations in our study. We studied a relatively small patient population. In addition, five of the patients were using \( \beta \)-blockers during the study period. This may have had an impact on the changes in HRV, but not on the changes in plasma noradrenaline and natriuretic peptide concentrations. In addition, no changes were made to the medications of the patients throughout the study. The use of vincristine may have had an influence on the changes in HRV, but not on natriuretic peptides or noradrenaline. Furthermore, in multivariate analysis, the changes in HRV were not associated with the cumulative dose of vincristine.

We conclude that doxorubicin-induced subclinical left ventricular dysfunction is characterized by very early sympathetic activation. During further progression of left ventricular dysfunction there is an attenuation of this sympathetic tone, accompanied by increased secretion of natriuretic peptides. An explanation for this decline in sympathovagal balance could be a sympatho-adrenal inhibition caused by increased secretion of natriuretic peptides.

REFERENCES


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