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THE CARDIAC MARKERS AND OXIDATIVE STRESS PARAMETERS IN ADVANCED NON-SMALL CELL LUNG CANCER PATIENTS RECEIVING CISPLATIN-BASED CHEMOTHERAPY

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Abstract

Introduction: Cardiotoxicity is a well known long-term consequence of lung cancer chemotherapy, however little is known about early subclinical changes in cardiac function.

Aim: The goal of the study was to assess early cardiotoxic effects of cisplatin-containing chemotherapy in stage III and IV lung cancer patients, measuring serum levels of selected cardiac markers in relation to oxidant effects.

Methods: We quantified the immediate impact of chemotherapy on cardiac troponin T (TnT), creatine kinase-myocardial band (CK-MB) and N- terminal pro-brain natriuretic peptide (NT-proBNP) in blood samples obtained from 12 non-small cell lung cancer (NSCLC) patients. All markers were measured using commercially available immunoassays. To investigate the oxidant effects of cisplatin-containing chemotherapy, we evaluated reduced glutathione (GSH), nitrite ($NO2^-$), derivatives of reactive oxygen metabolites (d-ROMs) and thiols (SH). Samples were collected prior to chemotherapy and 1 day after the first cycle of cisplatin administration.

Results: Chemotherapy did not cause statistically significant elevations in serum CK-MB. Serum TnT levels were undetectable at both time points in 11 out of 12 patients with a threshold of 0.01 ng/ml. In the single patient with undetectable TnT at the baseline, after the first infusion TnT level reversibly rose to 0.03 ng/ml. The pre-treatment value of NT-proBNP was slightly elevated in 7 out of 12 lung cancer patients. In 1 case NT-proBNP level significantly increased after chemotherapy (from 221.8 to 1489.0 pg/ml $p<0.001$), in the remaining 11 patients it was stable . Cisplatin-based combination chemotherapy induced significant nitrite production in 5 patients ($p<0.05$). The other measured oxidative stress parameters remained unchanged after the first infusion.

Conclusion: This pilot study demonstrated occasional elevations of cardiac biomarkers during cisplatin administration. Administration of cisplatin-containing chemotherapy caused significant nitroxidative stress in some patients. The

relevance of cardiovascular complications in cancer patients and identification individual risk factors of developing cardiovascular toxicity merit further evaluation.

INTRODUCTION

Lung cancer is the leading cause of cancer deaths worldwide. Cisplatin-based combinations are standard regimens in the treatment of advanced non-small cell lung cancer, significantly improving survival of patients. However, the high toxicity induced by cisplatin-based therapeutic regimens including ototoxicity, neurotoxicity, nephrotoxicity as well as cardiovascular damage limits their usefulness [1, 2, 3]. Cardiovascular complications resulting from conventional cancer chemotherapy are very heterogeneous. Several authors report changes in cardiovascular status within years to decades after chemotherapeutic treatment [3, 4, 5, 6, 7, 8], but little is known about the early changes in cardiac function in these patients. There is also evidence that oxidative stress is an important cause of chronic cardiotoxicity by cancer chemotherapy in humans [4]. The oxidative stress represents an imbalance between excessive generation and/or impaired removal of reactive oxygen species (ROS). In addition reactive nitrogen species (RNS) have a great potential to contribute to oxidative stress if they are overabundant [10, 11]. Thus excessive abundance of ROS and RNS with concurrent dysfunction of antioxidant defense systems, contributes to oxidative stress damage of the myocardium. Many chemotherapeutic regimens exert their effects, at least partly, through the oxidative pathway [4, 9, 10]. Cisplatin is known to accumulate in mitochondria and may induce ROS production by decreasing activity of antioxidants [2, 11, 12].

Cardiac blood biomarkers, obtained before and during chemotherapy, can be used to evaluate cardiac status and may help to identify patients at risk for cardiac toxicity [13, 14]. Several possible markers of cardiac damage have been identified. Since chemotherapeutic agent may cause disruption of cell membranes, resulting in the release of intracellular proteins such as lactate dehydrogenase (LDH), creatine kinase - myocardial band (CK - MB) and cardiac troponin (cTnT), these markers have been used to detect the presence and extent of myocardial injury [4, 13, 14]. Other potential markers include plasma levels of circulating natriuretic peptides, such as B-type natriuretic peptide, which is a marker of ventricular dysfunction and heart failure. N-terminal pro-BNP (NT-pro-BNP) is produced by ventricular cells in response to increased mechanical load and wall stretch [15]. It has been shown to be secreted from the cardiac ventricles in response to volume expansion and pressure overload [15]. Plasma levels of NT-proBNP are used as a prognostic indicator in different stages and causes of cardiac insufficiency [15]. Although cardiotoxicity is a known long-term consequence of lung cancer chemotherapy, little is known about early sub-clinical changes in cardiac function.

To address this issue, oxidative stress markers and cardiac markers were measured in non-small cell lung cancer patients treated with cisplatin-based chemotherapy prior to treatment and after first cycle of cisplatin administration.

PATIENTS

Twelve patients with median age 57 years, (range 48–70), with inoperable non-small cell lung cancer (stage IIIb and IV), scheduled to receive cisplatin-containing chemotherapy as first-line therapy at the Institute of Tuberculosis and Lung Diseases, Warsaw, Poland, between December 2006 and October 2007 were asked to participate in the study. Exclusion criteria were: age above 70 years at the start of chemotherapy, earlier radiotherapy, history of cardiac disease, serum creatinine level above 1.4 mg/dl, antioxidant therapy (vitamin C, E, herbal supplements, allopurinol or acetylcysteine). Chemotherapy consisted of cisplatin 80 mg/m^2 on day 1 and vinblastin 5 mg/m^2 on day 1 and 8. All regimens were administered through a peripheral vein. In addition every patient received 2 liters of isotonic fluids to prevent cisplatin-induced renal insufficiency and antiemetic treatment on day 1 of first cycle. Before treatment, all patients underwent a complete physical examination, chest radiography, ECG, chest computed tomography, urinalysis and blood tests. The study was approved by the Independent Ethics Committee and written informed consent was obtained from all participants

METHODS

The first blood sample was taken the day before chemotherapy, the second sample on the next day after cisplatin administration. All patients underwent routine laboratory testing at both time points along with cardiac and oxidative stress markers. The routine tests included: complete blood count, sodium, potassium, magnesium, calcium, creatinine, bilirubin, cholesterol and triglycerides, total serum proteins and D dimers. Plasma concentrations of all routine laboratory markers including total cholesterol, HDL cholesterol and triglycerides were determined by standard laboratory methods. The concentration of LDL cholesterol was calculated using the Friedewald equation, and expressed as mmol/l. Plasma magnesium concentrations were used as indicators of renal tubular function, as renal tubular magnesium wasting often occurs in patients treated with cisplatin. D-dimer measurements were performed using VIDAS (BioMerieux, Marcy L'Etoile, France) according the manufacturer's instructions. A D-dimer concentration of 500 ng/ml or less was defined as normal.

The cardiac markers analysis included: TnT, CK-MB mass and NT-proBNP. All markers were quantified using commercially available immunoassays on an Elecsys 2010 platform (Roche Diagnostics GmbH, Mannheim, Germany), according to established methods. Diagnostic thresholds for TnT, NT-proBNP and CK-MB were respectively < 0.01 ng/ml, 125 pg/ml, and 5.0 ng/ml as recommended. Total imprecision, as expressed by the coefficient of variation, was lower than 10% for all markers, as quoted by the manufacturer.

Examined oxidative stress markers included: reduced glutathione (GSH), nitrite (NO^{-2}), derivatives of reactive oxygen metabolites (d-ROMs) and thiols (SH). To measure oxidative stress markers 10 mL of venous blood was drawn into a tube containing ethylenediaminetetraacetic acid (EDTA). The sample was then centrifuged and plasma was separated. d-ROM's were measured in fresh plasma sample. The other measurement were performed in samples stored at − 70 °C until analysis.

d-ROM (mainly hydroperoxides) was measured immediately after blood collection. Plasmatic d-ROMs in presence of iron are able to generate alkoxyl and peroxyl radicals, according to the Fenton's reaction. Such radicals, in turn, are able to oxidize an alkyl-substituted aromatic amine, thus transforming them into a colored derivative photometrically quantified at 546 nm after 3 min of incubation at 37°C. A blank reagent obtained by replacing plasma with distilled water and a standard with assigned value was included for each series of assays. The intensity of the developed colour is directly proportional to the concentration of d-ROM, according to the Lambert–Beer's law.

Plasma nitrite (NO^{-2}) was determined by spectrophotometric assay based on the Griess reaction – a sensitive technique, which does not detect nitrate (NO^{-3}). The detection limit of the assay was 0.5 nmol/ml [16].

Total plasma thiols were determined using a spectrophotometric assay based on 2.2-dithiobisnitrobenzoic acid (Ellmann's reagent) according to the described method [17].

The concentrations of thiols, nitrite, d-ROMs and reduced glutation are expressed as µmol/L.

STATISTICAL ANALYSIS

Statistical analysis was carried out in the statistical software package Statistica. For comparisons of the results before and after cisplatin infusion non-parametric Mann–Whitney test was used. To calculate changes within a patient, Wilcoxon signed-rank test was used on the paired samples. *P*-values ≤ 0.05 were considered to indicate significant differences.

RESULTS

The pre-treatment values of NT-proBNP were slightly elevated in 7 NSCLC patients (Table 1). Although NT-proBNP levels varied markedly, no systematic changes were detected due to chemotherapy. In 1 case the level of this marker significantly increased after chemotherapy (from 221.8 to 1489.0 pg/ml, p<0.001) (Tab. 1). Serum TnT levels were undetectable at both time points in 11 of 12 patients with a threshold of 0.01 ng/ml (Tab. 2). In the single patient with

undetectable TnT at the baseline, after the first infusion TnT level rose to 0.03 ng/ml (Tab. 2), with no significant elevation detected after 1 week (data not shown). Similarly, no change in serum CK-MB levels was found at both time points (Tab. 1). Median levels of all three cardiac markers did not significantly increase after first infusion of cisplatin-based chemotherapy. At the baseline D-dimer was elevated in all patients except one. In 1 case D-dimer level increased significantly after cisplatin infusion, in remaining cases rested within the same range (Tab. 1).

The pre and post-treatment median levels of oxidative stress parameters did not significantly differed (Table 3). Cisplatin-based combination chemotherapy induced significant nitrite production in 5 patients ($p<0.05$) (Tab.3). The other measured oxidative stress parameters remained unchanged after the first infusion. (Tab. 3). Serum hemoglobin, urate, creatinine, bilirubin and serum proteins did not change after the first infusion of cisplatin (data not shown). Levels of cardiac markers did not correlate with oxidative stress parameters before and after treatment.

Table 1. Cardiac markers before (*) and after (**) first administration of cisplatin-based chemotherapy in non-small cell lung cancer patients.

Initials of the patient	ProBNP pg/ml*	Pro-BNP pg/ml**	CK-MB ng/ml*	CK-MB ng/ml**	DD ng/ml*	DD ng/ml**
S.E	268.2	140.7	1.0	1.1	2668	2590
J.Z	160.7	103.5	1.2	1.5	709	1190
G.H	139.1	202.6	2.6	2.4	506	502
L.S	50.6	40.3	1.0	1.5	539	607
D.I	169.2	187.8	5.2	5.5	544	518
Z.J	90.4	147.2	1.3	1.3	3546	5220
S.J	320.6	191.7	1.6	1.4	345	340
R.W	109.3	120.2	3.0	1.7	751	710
O.T	98.6	109.6	3.2	1.4	1729	1850
S.A	34.6	78	1.0	1.3	758	809
P.L	221.8	1489	1.6	1.4	7812	7875
B.M	291.5	283.1	2.4	2.4	693	647

Table 2 Troponin T before (*) and after (**) first administration of cisplatin-based chemotherapy in non-small cell lung cancer patients.

Initials of the patient	cTnT* ng/ml	cTnT** ng/ml
S.E	<0.01	0.03
others	<0.01	<0.01

DISCUSSION

Cardiac blood biomarkers have been extensively studied as long-term indicators of chemotherapy cardiotoxicity, with variable and contradictory results [13, 14, 15, 18, 19]. In contrast, very limited data exist regarding the immediate impact of administration of chemotherapeutic agents on cardiovascular status. In the present study we have evaluated the early_changes of serum levels of selected cardiac markers in relation to oxidant effects of cisplatin-containing chemotherapy in stage IIIb and IV non-small cell lung cancer patients. Examined patients were free from any known cardiovascular disease, however we could not exclude clinically silent myocardial damage as lung cancer and cardiovascular diseases share the same predisposing factors (i.e. cigarette smoking). Moreover 7 out of 12 patients presented with slightly to moderately elevated NT-proBNP level prior to administration of chemotherapy. The mechanism of this increase remains unresolved. Previous studies have shown that the level of natriuretic peptides become elevated before the development of chronic heart failure and even before a decline in ejection fraction occurs [20, 21]. Moreover, it is clear that elevation of natriuretic peptides does not unequivocally diagnose a cardiac disease, but it should be rather used in a more general way in order to detect possible cardiac involvement [20, 21].

Table 3. Oxidative stress markers before (*) and after (**) first administration of cisplatin-based chemotherapy in non-small cell lung cancer patients.

Initials of the patient	GSH*	GSH**	SH*	SH**	dROM*	dROM**	NO*	NO**
S.E	3.134	3.058	5.517	5.339	4.27	4.39	39.205	27.653
J.Z	2.619	3.188	6.249	6.432	3.609	3.397	54.728	95.626
G.H	2.397	2.298	5.917	6.461	3.561	3.628	84.708	83.838
L.S	3.103	3.103	5.032	6.78	4.209	4.234	21.166	10.685
D.I	3.663	3.587	7.158	6.502	2.397	2.652	8.451	32.471
Z.J	3.789	3.322	6.577	6.714	3.628	4.331	41.319	36.147
S.J	2.56	2.167	4.916	6.24	3.925	3.937	16.348	22.33
R.W	2.362	2.425	4.507	2.497	3.428	3.179	52.077	93.873
O.T	1.86	2.083	8.304	2.335	4.852	2.391	28.441	129.954
S.A	2.586	2.974	2.701	4.204	3.561	3.149	18.11	17.256
P.L	3.864	4.004	2.452	2.78	2.458	2.864	34.6	37.576
B.M	2.753	3.09	2.307	2.656	4.282	3.888	27.8	33.67

For example Fijalkowska et all. proved that NT-proBNP level increases with age up to 225pg/ml in elderly subjects without clinically overt cadiac disease [22]. The common conclusion of all of the systematic reviews is that low concentration of natriuretic peptide may be useful in ruling out the heart failure [20, 21, 23, 24]. A high plasma concentration of BNP is indicative for cardiac insufficiency in most cases, but occasionally NT-proBNP elevation is seen in the patients without the evidence of heart disease**,** nor provides specific information about any underlying cardiac abnormality [22, 24]. However it may have prognostic value. Bibbins et al suggest that in adults with coronary heart disease NT-proBNP levels predict cardiovascular morbidity and mortality and identify individuals at risk even in

the absence of cardiac dysfunction by echocardiography [23]. It remains unresolved why BNP concentration is high in some patients without heart disease. NT-proBNP is relatively dependent upon glomerular filtration rate, and circulating concentrations are typically higher in people with chronic renal disease [25, 26]. However, all patients with renal insufficiency were excluded from our study, additionally low glomerular filtration rate is a contraindication for cisplatin therapy. Some data suggests also that the hemodynamic changes that accompany anemia are sufficient to increase the synthesis of proBNP by cardiac myocytes as evidenced by increased concentrations of NT-proBNP [27]. It is possible that reduced Hb level in lung cancer patient contribute to the endocrine function of the heart by increasing the production of cardiac natriuretic peptides [27]. In one of our patients NT-proBNP level significantly rose after chemotherapy administration. It may be explained by cisplatin-induced cardiotoxicity resulting in reversible myocardial damage. On the other hand cisplatin administration was always accompanied by infusion of at least 2 liters of fluids. It may overload the circulation in the patients with occult cardiac insufficiency. Bibbins et al suggests that NT-proBNP levels predict cardiovascular morbidity and mortality in patients with coronary heart disease and identify individuals at risk even in the absence of cardiac dysfunction by echocardiography [23].

We have demonstrated that in one out of twelve patients TNT reversibly rose above the cut off level after the first cycle of chemotherapy. It is generally accepted that in the absence of myocardial injury, TnT levels are usually below the limit of detection of current analytical methods. The release of troponin into the circulation induced by other chemotherapeutics (anthracycline and doxorubicin) was associated with histological evidence of myocardial damage [24, 28]. The important point was that the prognosis of such patients was definitively worse compared to that of subjects in whom myocardial injury could be excluded using cardiac troponin measurement [29, 30]. Elevated troponin concentrations were found also in the absence of clinical evidence of ischemia, reaffirming the concept that troponin reflects rather myocardial necrosis but can not indicate its mechanism [29, 30]. Conditions like chemotherapy-induced cardiotoxicity can cause myocardial necrosis and, therefore, elevations in cardiac troponin level [7, 8, 14].

Our results do not eliminate the possibility that, in rare circumstances, cardiac biomarker elevations may be caused by cisplatin administration. However, if elevation is detected during treatment, further clinical investigation is warranted to discern alternative etiologies for the cardiac insult. Moreover alternative biomarkers will need to be identified to assess the risks of chemotherapy-induced cardiac toxicity.

Cisplatin is effective against a wide range of solid organ cancers [1, 2]. Its mode of action has been postulated to be its reactivity with the N7 position of guanine in the DNA chain leading to the formation of intra and interstrand crosslinks [31]. Previous studies showed that cisplatin-based chemotherapy produces oxidative stress, as evidenced by a decreased antioxidant [32]. Cisplatin has also been associated with the increased production of ROS, and biochemical manipulations aimed at reducing ROS production or detoxifying ROS have resulted in decreased toxicity of cisplatin [32, 33]. Our study concentrated on a solid tumor (lung cancer), and to our knowledge, only a couple of studies have examined the oxidative status during chemotherapy in lung cancer patients [34, 35].

Cardiotoxicity due to oxidative stress was attributed to various chemotherapeutics including adriamycin, cyclophosphamide, vincristine, doxorubicin [6, 7, 8, 18, 20, 33]. These drugs may increase free radical production and may also lower antioxidant enzyme concentrations in plasma during chemotherapy. Formation of free radicals, leading to oxidative stress, has been shown to be one of the side-effects of cisplatine [36]. Recently, it was demonstrated that plasma concentrations of various antioxidants decreased significantly during cisplatin-based chemotherapy in cancer patients [32, 36]. To assess oxidative stress we measured levels of reduced glutathione, derivatives of reactive oxygen metabolites, thiols and marker of nitrosative stress (nitrite) at baseline before treatment and after the first administration of cisplatin. In this study, no notable changes were seen in plasma level of GSH, SH and d-ROM after acute administration of cisplatin. Although Noori et al postulated that cisplatin-induced oxidative stress was connected with decreased level of tissue GSH, however this study differs from our observations in terms of the time point at which GSH was measured [32]. These authors observed effect of repeated treatment with high cumulative dose of the drug on rat model. Thus single administration of the drug may not be sufficient to

substantially change oxidative status in treated patient and it might take a longer time to observe the possible effect of chemotherapy on GSH level in serum. Reduced glutathione is a major intracellular redox buffer such that the ratio of reduced glutathione to oxidized glutathione can be used as a reflection of intracellular redox status [17, 32]. Thiols possess the ability to scavenge free radicals. Glutathione (GSH) is the most prevalent non-protein thiol in mammalian cells. GSH terminates free radicals with itself being oxidized to glutathione disulfide. Other authors observed increase in oxidized glutathione induced by combined therapy including cisplatinum [2, 4, 33]. However this effect can be probably seen during long-term drug administration. Further studies will determine if this is indeed the case.

In our study, a significant increase of nitrite level was observed in some of lung cancer patients as the immediate effect of cisplatin administration. Nitrite in plasma reflect the levels of nitric oxide, a multifunctional molecule involved in a variety of physiological and pathological processes [10]. In the presence of superoxide, nitric oxide spontaneously forms peroxynitrite. Peroxynitrite is much more reactive than superoxide and nitric oxide and can exert direct oxidative modifications. It has an important role in the initiation of apoptosis in various cell types in clouding cardiomyocytes [10, 37]. In the present study, the levels of nitrite increased during the course of chemotherapy treatment in 5 out of 12 patients. Similar observations confirmed by Zhou et al in a clinical study, the post-chemotherapy serum NO level was significantly higher than that found before chemotherapy in responsive patients with lung cancer [38]. Also Chirino et al demonstrated that an increased peroxynitrite generation is involved in cisplatin-induced nephrotoxicity [2]. In addition, Deng et al demonstrated an increase in iNOS mRNA levels in kidney 4 h after cisplatin administration [39]. Opposite Colakogullari et al did not confirm our observation as they did not find any alteration in serum concentrations of nitrite/nitrate 24 and 48 h after the first cisplatin-based chemotherapy course [40]. The difference can be attributed to the methodological aspects. This author also found that high production of NO was associated with a high risk of poor survival [40]. The survival rate was 8 times lower compared with the patients who had lower serum NO levels. No data is provided at present on the role of NO in cisplatin-induced cardiotoxicity. It has been found that NO and it's metabolites including peroxynitrite are toxic [2, 12]. Despite of its non-radical nature, peroxynitrite is more reactive than their parent molecules O_2^- and NO. It is well known that peroxynitrite may injure physiological cell processes by initiating lipid peroxidation, producing DNA breakage, reacting with thiols, and causing inhibition of mitochondrial respiration [2, 12]. The peroxynitrite generation could also inactivate enzymes and ion channels via protein oxidation on methionine, cysteine, tryptophan or tyrosine residues and by nitration of tyrosine or tryptophan residues [2, 12]. It has been shown that cisplatin administration induced a large amount of NO which may lead to ROS and RNS greater formation causing cellular damage. [12]. A toxic role for nitric oxide itself or by generation of nitrosative stress has also been described [2, 12]. There are evidences that peroxynitrite formation contributes to renal damage in nephrotoxicity induced by cisplatin administration [2, 12]. Our observations support the hypothesis of excessive NO, occurring in some patients after cisplatin administration. Whether this mechanism contribute to the pathogenesis of cisplatin-induced cardiotoxicity remains unknown. Induction of cell death by apoptosis, cytoskeleton derangement, F-actin damage by cisplatin has also been associated with cisplatin administration [41, 42]. Attempting to explain the cisplatin-associated induction of apoptosis, various authors have established its interaction with DNA, which produces a complex that inhibits DNA replication and transcription and impairs DNA damage repair [39]. Additionally, cisplatin was also found to inhibit protein synthesis and/or induce mitochondrial injury, finally leading to apoptosis [2, 12, 41, 42]. Several authors suggest that mitochondria might be the initial event causing cisplatin-induced injury [2, 12, 42]. Mitochondrial nitric oxide synthase can be one of the major sources of peroxynitrite generation leading to apoptosis promotion in ischemia-reperfusion (I/R) injury [42, 43]. The most important question to be addressed is whether over production of NO metabolites (like peroxynitrite in tissue, nitrite/nitrate in the blood stream) damage the vital organs like the heart, shortening the life span of the patients. Therefore, its possible prognostic significance deserves to be elucidated using larger groups over a longer term.

CONCLUSION

Our data demonstrate that elevations in TnT and NT-proBNP occur occasionally during cisplatin administration in non-

small cell lung cancer patients and if detected, require further clinical evaluation. As chest pain, dyspnea and cough are common in patients with thoracic malignancies, cardiac blood biomarkers may be employed to investigate these symptoms. Immediate effect of administration of cisplatin-containing chemotherapy was significant nitrosative stress as implied by increased levels of nitrite in plasma. Other examined oxidative stress markers seem to behave differently. The further investigations are needed to clarify the relevance of cardiovascular complications in cisplatin treated lung cancer patients, and to identify individual risk factors of developing cardiovascular toxicity. In addition, there is a need to identify early signs of cardiac damage in order to optimize the clinical management of cardiotoxicity. The extreme variety of clinical phenotypes of cardiovascular complications in cancer patients underlines the importance to clarify the primary mechanisms of cardiotoxicity of anticancer drugs and to understand the possible interactions in combination therapies. Effective markers of acute cardiac damage during treatment could predict long-term cardiac outcomes and allow modification of the treatment protocol.

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