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The cytotoxic drug cyclo-pentenyl cytosine: from manufacturing to anti-tumor activity and (cardio)toxicity
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CHAPTER 8

DOXORUBICIN AND CPEC INDUCED CARDIOXICITY: association with the GTPase gene Rac2, and drug transporter genes MRP1 and MRP2

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ABSTRACT

Chemotherapy with anthracyclines is associated with cardiotoxicity. Besides known risk factors such as cumulative dose and mediastinal irradiation, there are indications that genetic variation might also be associated with the development of anthracycline induced cardiotoxicity. In the current study a retrospective case control analysis was performed in oncology patients having received anthracyclines. Allele frequencies of genetic polymorphisms in several candidate genes between patients with and without anthracycline-induced cardiotoxicity and between cases and healthy control subjects were studied. Anthracycline-induced cardiotoxicity was associated with the T7508A variant in the GTPase Rac2, a gene with a known functionality in NAD(P)H oxidase activity. Furthermore, we tested *in vitro* in a rat cardiomyocyte cell line (H9c2), whether the anthracycline doxorubicine and the cytotoxic and cardiotoxic drug cyclopentenyl cytosine (CPEC) influenced the expression of the candidate genes (p22phox, Rac2, MRP1 and MRP2), that were reported earlier to be related with doxorubicin-induced cardiotoxicity. CPEC caused a small decrease in the expression of MRP2 and did not influence the expression of the other genes. The expression of MRP1 was decreased after incubation with doxorubicin whereas no changes were found in the expression of Rac2, p22phox and MRP2.

It is concluded that although the expression of Rac2 did not seem to be influenced by doxorubicin, a SNP in the GTPase Rac2 was associated with anthracycline induced cardiotoxicity. As demonstrated by their influence on MRP1 and MRP2 respectively, doxorubicin and CPEC decreased the expression of genes involved in multi drug resistance.

INTRODUCTION

Cardiotoxicity is associated with several cytotoxic drugs, among which the anthracyclines seem to be the most important group and much research has been done with anthracyclines to unravel the mechanisms behind this severe side effect [1-4]. Oxidative stress caused by reduction of doxorubicin seems to play an important role in the pathogenesis. Several risk factors such as age, mediastinal irradiation and cumulative dose have been associated with anthracycline induced cardiotoxicity. Of these, cumulative dose seems to be the most important risk factor [5,6]. The incidence of doxorubicin induced cardiomyopathy and congestive heart failure increases from approximately 3-5% below 400 mg/m^2 to 20-50% at 700 mg/m^2 [7,8]. A maximum cumulative dose of 450-550 mg/m^2 for doxorubicin is nowadays commonly accepted. Depending on their cardiotoxic potential, maximum cumulative doses are also established for the other anthracyclines [9]. However, as indicated by the 5% of patients with cardiotoxicity having received 400 mg/m^2 doxorubicin or less, there is a category of patients still developing cardiotoxicity upon low to normal cumulative doxorubicin doses.

These inter-individual differences may have a genetic cause and several mechanisms are conceivable that could explain this relationship. One mechanism might be found in the oxidative stress theory. As oxidative stress seems to play an important role in the development of the anthracycline induced cardiotoxicity [1-3], changes in enzymes involved in oxidative stress may result in more or less susceptibility for anthracycline induced cardiotoxicity. The NAD(P)H oxidase multi-enzyme complex is an important source of reactive oxygen species in the myocardium [10]. Indeed, recently Wojnowski *et al* reported associations between polymorphisms in the NAD(P)H oxidase multi-enzyme complex and cardiotoxicity of doxorubicin [11]. They used a candidate gene approach to detect associations between cardiotoxicity and single nucleotide polymorphisms (SNPs). Besides the three SNPs in genes related to the NAD(P)H oxidase complex (p22phox, p40phox and Rac2), two SNPs in genes coding for the drug efflux pumps MRP1 and MRP2 (multidrug resistance protein 1 and 2) were also associated with doxorubicin induced cardiotoxicity. Anthracyclines are known substrates for the efflux transporter MRP1 [12,13], and polymorphisms of this pump are described in other studies [14,15].

Apparently the five genes studied by Wojnowski *et al* are involved in the mechanisms behind doxorubicin induced cardiotoxicity. However, NAD(P)H oxidase and the efflux pumps both are not exclusively related to the function or metabolism of doxorubicin. It is therefore conceivable that changes in these genes are also related to cardiotoxicity induced by other cytotoxic drugs. The experimental cytotoxic drug cyclopentenyl cytosine (CPEC) has also been associated with cardiotoxicity and until now the mechanism has not been clarified [16, 17]. CPEC is a nucleoside analogue structurally related to the cytotoxic drugs cytarabine and gemcitabine. These drugs are not thought to be substrates nor modulators for MRP1. Substrates for MRP2 are supposed to be similar to those for MRP1, however, there is little information available upon modulators for

MRP2 [18]. If CPEC would be able to modulate MRP2 by altering its expression, this might be involved in CPEC induced cardiotoxicity.

In the present study we aim to replicate the results of the study by Wojnowski *et al* in a case control study. Moreover, in order to further investigate the association of the candidate genes and cytotoxic drug induced cardiotoxicity, we have also investigated in a rat cardiomyocyte cell line (H9c2), the expression of the candidate genes upon incubation with the anthracycline doxorubicin and the experimental cytotoxic drug CPEC.

MATERIAL AND METHODS

Study population

We retrospectively selected study subjects by reviewing all patients with cancer (n=1074) that received doxorubicin between january 1999 and april 2005. Patients were included in this study if they were subjected to routine cardiac assessment (either by left ventricular ejection fraction (LVEF) measurement or echocardiography) and if DNA was available. Cases could also be directly selected by the participating physicians if the patients were known for clinically overt anthracycline induced cardiotoxicity. Patient material was obtained from blood samples from routine control and was coded before analysis in order to prevent results from being related to individual patients. The protocol was approved by the institutional Medical Ethical Committee.

Based on the described selection a group of cases (n=11) and the first control group (n=26) were determined. The first control group consisted of patients without reported cardiotoxicity, for criteria see below. Most patients had received doxorubicin, if patients had received another anthracycline (2 of the cases: one received daunorubicin and mitoxantrone, the other received doxorubicin and daunorubicin, 1 control patient received epirubicin and doxorubicin), the received cumulative dose of that anthracycline was converted to the equivalent cumulative doxorubicin dose. The conversions were based on the different maximal cumulative dosages which were respectively 450-550 mg/m^2 for doxorubicin, 500-600 mg/m^2 for daunorubicin, 850-1000 mg/m^2 for epirubicin and 160-200 mg/m^2 for mitoxantrone [9]. For example, 420 mg of epirubicin was converted to 227 mg of doxorubicin (420 * 500/925 = 227). Patients having received more than 550 mg/m^2 of cumulative doxorubicin dose were excluded from the analysis.

The second control group consisted of a panel of 180 healthy volunteers. The DNA of these subjects was previously selected in families of patients who had had meningococcal disease[19] and in subjects who served as healthy controls to study the susceptibility to systemic lupus erythematosus [20].

Cardiotoxicity assessment

Cardiotoxicity was determined for the group of cancer patients and was based on the following criteria: clinically overt cardiotoxicity or a decrease in left ventricular ejection fraction (LVEF) of more than 20% or a decrease of more than 10% resulting in a LVEF below 50% [8]. If assessment of cardiac condition was performed by echocardiography, cardiotoxicity was defined as a decrease of more than 10% or a decrease resulting in a shortening fraction (SF) below 30% [21].

Sample preparation and measurement of SNPs

Six SNPs in five candidate genes were selected that had been associated with doxorubicin induced cardiotoxicity in a previous study [11]. Three selected genes had a relation with the NAD(P)H oxidase multi-enzyme complex: those encoding for the GTPase Rac2, the subunit p40phox (NCF4) and the subunit p22phox (CYBA) from the NAD(P)H oxidase multi-enzyme complex. The other genes were encoding for MRP1 and MRP2. The following SNPs were analyzed: p40phox 212A>G (rs1883112), p22phox His72Tyr (rs4673), Rac2 7508T>A (rs13058338), MRP1 Gly671Val (2012G>T), MRP2 Val1188Glu (rs8187694), MRP2 Cys1515Tyr (rs8187710). The SNPs for p22phox, MRP1 and MRP2 were non-synonymous. The genes encoding for Rac2 and NCF4 were both found on chromosome 22, p22phox and MRP1 on chromosome 16 and MRP2 on chromosome 10.

Table 1 primer sequences used for SNP assay

Name	primer direction	sequence 5'-3'
NADPH-f	forward	GCAAAGGCTTGGCAGTAAGAGAA
NADPH-rBio	reverse	CAAGCCTCAGTTGGGTATCAGAA*
NADPH-fs	forward	GGTCACAAGACA
P22-f	forward	GCAAAGGAGTCCCGAGTG
P22-rBio	reverse	GTAAAGGGCCCGAACAGC
P22-fS	forward	CCCCAGGGGACAG
Rac2-fBio	forward	CCCCAGCACCCAGGTATCA
Rac2-r	reverse	TTGCCCTGAGAACCAAGACCT
Rac2-rS	reverse	TCTGGGTTCCTTGAATGC
MRP2-val1188glu-f	forward	AGCACCAGCAGCGATTTC
MRP2-val1188glu-rBio	reverse	GGAGGTGATCCAGGAAAAGAC
MRP2-val1188glu-fS	forward	GATTTCTGAAACACAATG
MRP2-cys1515tyr-fBio	forward	ATGGTCCTAGACAACGGGAAGAT
MRP2-cys1515tyr-r	reverse	GCCTTCTGCTAGAATTTTGTGC
MRP2-cys1515tyr-rS	reverse	TCTTCAGGGCTGCCG
MRP1-f	forward	GAGCGACCCTCCCACACT
MRP1-rBIO	reverse	ACTTTCCGCAGCCCACCT
MRP1-fs	forward	TCTCCATCCCCGAA

* Primers are Biotine labeled at 5’-end

DNA was isolated from peripheral blood cells by a standard manual salting-out method. Sterile water was used as a negative control. Genotyping was performed using real-time polymerase chain reaction with TaqMan (MRP1) (Applied Biosystems, Nieuwerkerk aan de IJssel, The Netherlands) or Pyrosequencer (Isogen Lifesciences, Maarssen, The Netherlands) (other SNPs) according to manufacturer's instructions. In table 1 the sequences of the primers are displayed.

Statistical analysis

Differences in baseline characteristics between the cancer patients were analyzed by Student's t-test (continuous variables) or chi-square test (dichotomous variables). P values less than 0.05 were considered significant. For association with cardiotoxicity, differences in genotype distribution were tested by 3 x 2 cross tabulations for each genotype with analysis by Fisher's exact test, and by 2 x 2 cross-tabulations for allele carriers versus noncarriers with calculation of the odd's ratios (95% confidence interval). Binary logistic regression with mediastinal irradiation as a covariate was used to test whether mediastinal irradiation had to be considered as a possible confounder.

All statistical analysis were performed using SPSS 12.0.1 software (SPSS, Chicago, USA).

Cellular experiments

The rat cardiomyocyte cell line H9c2 (ECACC, Wiltshire, UK) was used for in vitro assays. This cell line has been used before to study anthracycline induced cardiac injury [22-24]. The ability of doxorubicin and CPEC to change the expression of the candidate genes was explored. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Cambrex Corporation, East Rutherford, NJ, USA) containing 2mM L-glutamine, 100 IU/ml penicillin G, 100 μg/ml streptomycin and 10% fetal calf serum (Invitrogen, Breda, The Netherlands). Cells were not allowed to reach more than 70% confluency.

For determination of cytotoxicity cells were plated in 96-well plates (approximately 20 x 10^3 cells per well). After 24 hours incubation at 37ºC in a humidified environment of 5% CO_2, cells were exposed to different concentrations of doxorubicin (0 – 10 μg/ml) (Pharmachemie, Haarlem, The Netherlands) and CPEC (0-1 μg/ml) (kindly provided by the National Cancer Institute, Maryland, USA) for 24 hours. After incubation with the active ingredient, medium was replaced and cells were further cultured for 72 hours. Cytotoxicity was determined by the sulforhodamine B (SRB) assay as described earlier [25,26]. Briefly, after drug treatment, cells were fixed with 100 μl thrichloroacetic acid (10%) for 60 minutes at 4ºC, washed with water and stained with 100 μl SRB (0.4% in 1% acetic acid) for 30 minutes at room temperature. Then plates were rinsed with acetic acid (1%) and 200 μl Tris base (10mM, pH 10.5) was added. Optical density was determined at 560 nm in each well in a microplate reader (Softmax, Biorad, Veenendaal, The Netherlands).

For the expression assays H9c2 cells were treated with doxorubicin 1 μg/ml or CPEC 1 μg/ml for 4 or 24 hours (in triplo). At these concentrations approximately 90% of the cells were still viable during the incubation time (4 to 24 h). Total RNA was isolated from the cells using the mini RNeasy total RNA isolation kit (Qiagen, Venlo, The Netherlands) and the protocol supplied by the manufacturer. The isolated RNA was converted to cDNA using standard techniques. cDNA of Rac2, p22phox, MRP1 and MPR2 were analyzed by RT-PCR on the Taqman 7500 in duplo using SYBR green (Applied Biosystem, Nieuwerkerk aan de IJssel, The Netherlands). The sequences of the used primers are described in table 2. The expression of β2-microglobuline gene was used as an internal control, which had been proven to be stable expressed in these experiments.

The relative changes in gene expression were calculated with the $2^{-\Delta\Delta Ct}$ method With this method data are presented as the fold change in gene expression normalized to the endogenous control gene (β2-microglobuline) and relative to the untreated control at the same time interval. The range in fold change is determined by calculating respectively the fold change out of the average ΔCt + SD and ΔCt – SD [27].

Table 2: primer sequences used for expression assay

Name	primer direction	sequence 5'-3'
MRP1-f	forward	GGTCAGCCCGACACTGCTA
MRP1-r	reverse	TCCTTCGCTCAAACTGAATTAAAA
Rac2-f	forward	CAGACGTGTTCCTCATCTGCTT
Rac2-r	reverse	CCACTTGGCTCGGACGTT
MRP2-f	forward	CGTCTCCTACGGTTTCCAGATT
MRP2-r	reverse	GGAGTTTGTGTTGAGTCACTTGGT
P22-f	forward	CTCTATTGTTGCAGGAGTGCTCAT
P22-r	reverse	GGTGGAGCCCTTTTTCCTCTT

RESULTS

Patients

The characteristics of the cases and the first group of control (cancer) patients are represented in table 3. Cases were (non significantly) more frequently irradiated in the mediastinal region. However, when mediastinal irradiation was used as a covariate in the statistical analyses, this was of no relevance for the odd's ratios for the calculated differences between cases and controls for the SNPs that were analyzed. Therefore, we concluded that mediastinal irradiation did not confound our analysis.

Table 3 Patient characteristics

	cases (n=11)	controls (n=26)
Gender, M/F	1/10	13/13
Age in years(SD)	36 (19)	42 (16)
Range in age	13-69	21-73
Cumulative doxorubicin dosein mg/m2 (SD)	399 (93)	332 (113)
Mediastinal irradiation	5	6
Disease		
Sarcoma*	6	13
Mamma carcinoma	2	3
Acute Myeloid Leukemia (AML)	2	0
Non Hodgkin Lymphoma (NHL)	1	3
Hodgkin Lymphoma (HL)	0	6
M Kahler	0	2

*different forms of sarcoma's: ewing, osteo, synovium, bone-leiomyo

There were no statistic differences between the two groups in age, cumulative dose or mediastinal irradiation.

No significant associations with cardiotoxicity were found for the p40phox 212A>G, p22phox His72Tyr, MRP1 Gly671Val, MRP2 Val1188Glu and MRP2 Cys1515Tyr polymorphisms between either cases and control patients or healthy subjects. Combinations of SNPs based on either the location on the same chromosome (Rac 2 with p40phox, p22phox with MRP1 and both SNPs in MRP2) or physiological role (Rac2, p40phox and p22phox) neither showed a significant association with cardiotoxicity.

A significant association with cardiotoxicity was found for the SNP in Rac2. Frequency of the A allele was significantly lower in healthy subjects compared to cardiotoxic cancer patients (35% versus 82%) with an odds ratio of 0.12 (0.03-0.58). Distribution in the control patient group was in line with this observation (57% versus 82%), although it reached not significance (odds ratio 0.29, range 0.05-1.65).

Cells

In table 4 the relative fold expression changes with their ranges are presented for the genes studied. The expression of MRP1 was approximately 3 fold decreased upon 24 hr exposure of doxorubicin (mean fold change 0.32, range 0.18-0.57), see figure 1. No changes in expression were seen after 4 h of incubation. The expression of the other genes remained unchanged after treatment with doxorubicin both after 4 and 24 h of incubation. Although the range in fold change was relatively large, CPEC seemed to decrease the expression of MRP2 after a 24 h incubation period (mean 0.49, range 0.25-0.85), no changes were seen after 4 h of incubation. CPEC did not induce changes in expression of the other genes (4 and 24 h of incubation).

Table 4 Relative fold changes in expression upon exposure to doxorubicin or CPEC

	normalized fold change ($2^{-\Delta\Delta Ct}$) relative to untreated control (range)			
	Doxorubicin 24 hr	Doxorubicin 4 hr	CPEC 24 hr	CPEC 4 hr
MRP1	0.32 (0.18-0.57)	0.79 (0.31-1.45)	0.81 (0.39-1.30)	0.94 (0.34-2.30)
MRP2	0.55 (0.23-1.09)	0.68 (0.28-1.12)	0.49 (0.24-0.85)	0.98 (0.46-2.34)
Rac2	0.61 (0.23-1.14)	0.71 (0.19-1.77)	0.54 (0.21-1.16)	0.88 (0.27-2.93)
P22phox	0.85 (0.28-1.27)	1.14 (0.45-2.37)	1.03 (0.53-1.80)	1.11 (0.56-2.5)

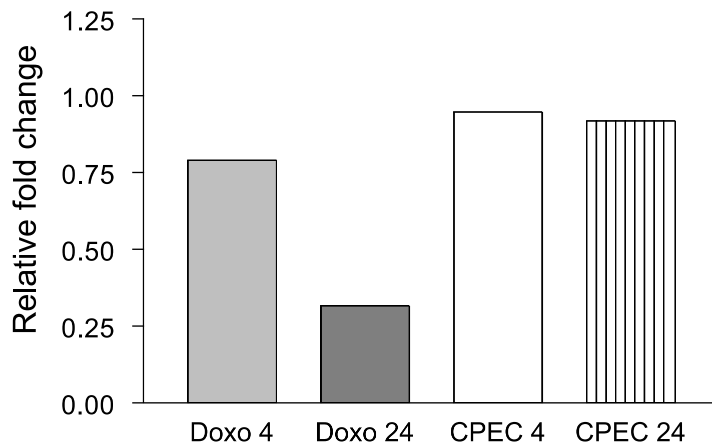

Figure 1 Fold change in expression of MRP1 relative to untreated samples

In the figure the fold change in expression of MRP1 relative to the untreated samples is presented. The four bars indicate the different treatments (e.g. doxo 4 means treatment with doxorubicin for 4 h). After 24 h of incubation with doxorubicin the expression of MRP1 is approximately 3 fold decreased compared to the untreated samples.

DISCUSSION

In the present study we have used two approaches to investigate the association between five candidate genes (p22phox, p40phox, Rac2, MRP1 and MRP2) and cytotoxic drug induced cardiotoxicity. First we studied SNPs in these genes in a retrospective case control study and secondly we investigated whether the expression of these genes changed upon incubation with doxorubicin or CPEC.

The five candidate genes were selected based on a study by Wojnowski *et al.* In a population of non-Hodgkin patients they found an association between 6 SNPs in five genes related with either NAD(P)H oxidase enzyme complex (p22phox, p40phox and Rac2) or multidrug efflux pumps (MRP1 and MRP2) and doxorubicin induced cardiotoxicity [11]. There is very little information on the functional analysis of the SNPs studied. For the SNP in p22phox (CYBA) conflicting results regarding its influence on NAD(P)H oxidase are published [28,29]. The SNP in MRP1 is located near the 'Walker A motif' and studies suggest that mutations near this motif would be able to decrease the activity of the efflux pump [14,15]. For the other SNPs no data in the published literature are available.

In our study we have been able to reproduce the association as reported earlier by Wojnowski *et al* between the SNP in the Rac2 gene in cancer patients experiencing anthracycline induced chronic cardiotoxicity when compared with a healthy population.

No difference between cancer patients with and without cardiotoxicity was found in our study, although the difference in allele frequency observed for Rac2 was in line with the observed statistical difference between cases and the healthy population. There were no statistical differences between the cases and the patient control group in the known risk factors cumulative dose and mediastinal irradiation. Several patients had received cyclophosphamide which has also been associated with cardiotoxicity, but there was no statistical difference between the two groups. Moreover, little information is known about prevalance, risk factors and mechanism of cyclophosphamide induced cardiotoxicity [6]. There were significantly more female patients in the case group, however, gender is not a known risk factor for anthracycline induced cardiotoxicity.

We did not observe associations between the other SNPs and cardiotoxicity. Our patient population probably was too small to detect differences as reported by Wojnowski et al [11]. In that study 83 patients with doxorubicin induced cardiotoxicity were evaluated whereas in the present study 11 cases were included. However, the cases in our study were selected out of a group of 1074 patients treated with anthracyclines in our large academic medical center in a 6.5 years period, indicating the low frequency of overt anthracycline induced cardiotoxicity. Indeed, a crude incidence of approximately 1.1% of anthracycline induced cardiotoxicity can be estimated from our cohort which is in line with published incidence rates being approximately 3-5% [7,8]. This observation also implicates that, when implementing (with the current knowledge) a diagnostic screening aimed at detecting patients at increased risk for anthracycline induced cardiotoxicity, would result in an unrealistically high number needed to genotype.

Besides the analysis of the SNPs, our second approach to investigate the association between the candidate genes and cardiotoxicity consisted of determining the influence of the cytotoxic drugs doxorubicin and CPEC on the expression of the genes. As little information on the functional analysis of the SNPs is yet available, this might help to clarify the role of the genes and the specific SNPs.

The NAD(P)H oxidases are involved in the formation of reactive oxygen species. More NAD(P)H activation leads to more superoxide formation and this may result in increased cardiotoxicity. Rac2 is essential for stimulation of the NAD(P)H oxidase [30] and is mainly expressed on cells from hematopoietic system, however the Rac2 protein has also been detected on rat enterocytes [31] and human aortic smooth muscle cells [32]. A decreased expression of Rac2 may result in a less activated NAD(P)H oxidase. Wojnowski *et al* have shown that mice with reduced NAD(P)H oxidase activity experienced less cardiotoxicity after treatment with doxorubicin [11]. The protein p22phox (CYBA) is necessary in NAD(P)H activation [33,34]. Based on their different roles, an increased expression of Rac2 (SNP rs13058338) and p22phox (SNP 4673) upon incubation with doxorubicin might be expected. However, we did not reveal any significant changes in the expression of both genes upon exposure to doxorubicin and CPEC when compared to the untreated cells. Rac2 was weakly expressed (Ct values between 30 and 34) in the H9c2 cells, which might

explain the relatively large range in fold change. However, p22phox had a higher expression (Ct values between 22 and 24) and the range was still relatively large suggesting that other influences might have played a role as well.

Expression of multidrug resistance proteins in tumor cells is associated with resistance to anthracyclines [18,35]. An impaired function of MRP1 might be associated with higher anthryacycline levels in the heart, rendering it more susceptible to the detrimental effects of anthracyclines. The reduced expression of MRP1 that was detected in our study after 24 hr incubation with doxorubicin seems to confirm this hypothesis. Apparently doxorubicin needs time to exert its influence on the expression of MRP1, as no difference with the untreated samples was detected after 4 h of incubation. Earlier reports on human small cell lung cancer cell lines [36] and heart tissue of mice [37], showed an increase in MRP1 expression after treatment with doxorubicin. In the study with the human small cell lung cancer cell line, c-jun N-terminal kinase activation (JNK) seemed to be essential for the induction of MRP1 by doxorubicin [33]. It is conceivable that JNK acts differently in H9c2 cells. The fact that the results of the two studies were obtained in different tissues or species might therefore have been responsible for the apparently conflicting results on MRP1 expression by doxorubicin.

CPEC did not influence the expression of MRP1, which might be partly explained by its chemical structure. CPEC is a nucleoside analogue and has structural similarities with the cytotoxic drugs cytarabine and gemcitabine and both drugs are not thought to be either substrates nor modulators of MRP1 [18]. MRP2 was slightly downregulated after incubation with CPEC and although substrates for MRP1 also are suggested to be substrates for MRP2, there is no information available upon modulators of MRP2 [18].

In this study we evaluated the abilities of doxorubicin and CPEC to influence the expression of four selected genes. However, doxorubicin has also been reported to induce the expression of several other genes involved in heart function like endothelial nitric-oxide synthese (eNOS), atrial and brain natriuretic peptide (ANP and BNP) and Fas antigen, the latter being involved in apoptosis [38-40]. Based on the structure of CPEC it might have been interesting to investigate the influence on MRP4 and MRP5 as they are believed to be involved in nucleoside transport [18].

Moreover, it remains to be solved whether the induced damage by doxorubicin or CPEC is related to altered gene or whether they directly influence the expression. This illustrates the complexity behind the induced cardiotoxicity and changes in expression of specific genes may only partly explain the mechanism.

In conclusion, in our small patient population, we have been able to detect an association between anthracycline induced cardiotoxicity and a polymorphism in the GTP-ase Rac2 which is associated with the function of the NAD(P)H oxidase complex. This is in line with the hypothesis regarding

the involvement of oxidative stress in the development of anthracycline induced cardiotoxicity. Although we did not find an association between SNPs in the efflux pumps and cardiotoxicity, the results of our expression study show a downregulation of MRP1 in rat cardiomyocytes after incubation with doxorubicin and a small decrease in expression of MRP2 after incubation with CPEC, suggesting that increased drug exposure of heart cells to doxorubicin or CPEC might also be involved in the complex mechanism of this severe side effect.

Overall, these results suggest that genetic analysis of SNPs or genes might clarify some of the issues, but will probably only partly explain cytotoxic drug induced cardiotoxicity.

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