Does the Multidrug-Resistance Modulator Cyclosporin A Increase the Cardiotoxicity of High-Dose Anthracycline Chemotherapy?

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Cyclosporin A has heterogeneous effects on anthracycline-related cardiotoxicity and can prevent multidrug-resistance (MDR). The aim of this study was to explore whether the coadministration of cyclosporin A is accompanied by an increase in cardiotoxicity. Forty-three patients (27 male, 16 female, age: 18–67 yrs (mean: 47.5 yrs, SD: 11.6 yrs)) received 177 radionuclide ventriculography examinations (RNV/177 at rest, 133 at stress) before and during chemotherapy with either doxorubicin (n = 23) or epirubicin (n = 20). RNV studies were applied up to 11 times in the follow-up of the patients. A maximum of 10 courses of chemotherapy was performed. In the doxorubicin group only, the age of the patients and the cumulative dose of the chemotherapeutic agent had a significant negative impact on left ventricular ejection fractions, whereas cyclosporin A had a significant positive influence (multiple analysis of regression, $p < 0.05$). Cyclosporin A did not cause any significant increase in cardiotoxicity in our patients.

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Anthracyclines are established chemotherapeutic agents with a high antitumorous potential, but in clinical use they have several limitations such as cardiotoxic side effects. The biological action of the anthracyclines includes inhibition of the mitochondrial oxidative phosphorylation, the DNA and RNA polymerases and DNA repair enzymes, the metallothionein synthesis, the topoisomerase II, and the helicases. Further effects are known by the production of free radicals, membrane modulation, and endonucleolytic cleavage (1).

The cardiotoxicity of doxorubicin, in particular, depends on a major metabolite, the 7-deoxy aglycone (2), which causes a pathologic increase in the calcium permeability in the liver mitochondria. Although cyclosporin A is known to inhibit this process, this may not result in a decrease in the doxorubicin-related cardiotoxicity, because, on the other hand, high doses of cyclosporin were found to cause severe myocardial calcification and fibrosis (in mice) (3).

A decrease in the anthracycline-related antitumor effects may be due to a so-called 'multidrug-resistance' (MDR) which corresponds to an export of chemotherapeutic agents by the tumor cells. The MDR may be present prior to the chemotherapeutic treatment or can occur after repeated administrations of anthracyclines. The classical MDR is related to the majority of anthracyclines, the vinca alkaloids, the podophyllotoxins, actinomycin D, mitoxantrone and the taxanes (1). In animal studies it has been suggested that a membrane glycoprotein (Pgp, synonym: P170) pumps the drugs out of the cells as the underlying principle of the MDR (1). The immunomodulator cyclosporin A is known to overcome MDR, possibly due to its potential to bind to Pgp (1).

Because the reported effects of cyclosporin A on the cardiotoxicity seemed to be rather contradictory, the aim of this study was to investigate whether the coadministration of cyclosporin A is accompanied by an increase in anthracycline cardiotoxicity in clinical practice.

MATERIAL AND METHODS

Patients

The study comprised 43 patients (27 male, 16 female), aged 18–67 years (mean: 47.5 yrs, SD: 11.6 yrs). Twenty patients had sarcomas, 11 carcinomas, and 12 had other neoplasias. The patients were divided into two groups, one receiving doxorubicin (n = 23), the other epirubicin (n = 20).

Methods

Chemotherapy. Owing to the varying tumor entities, the chemotherapy scheme was not quite uniform in the pa-

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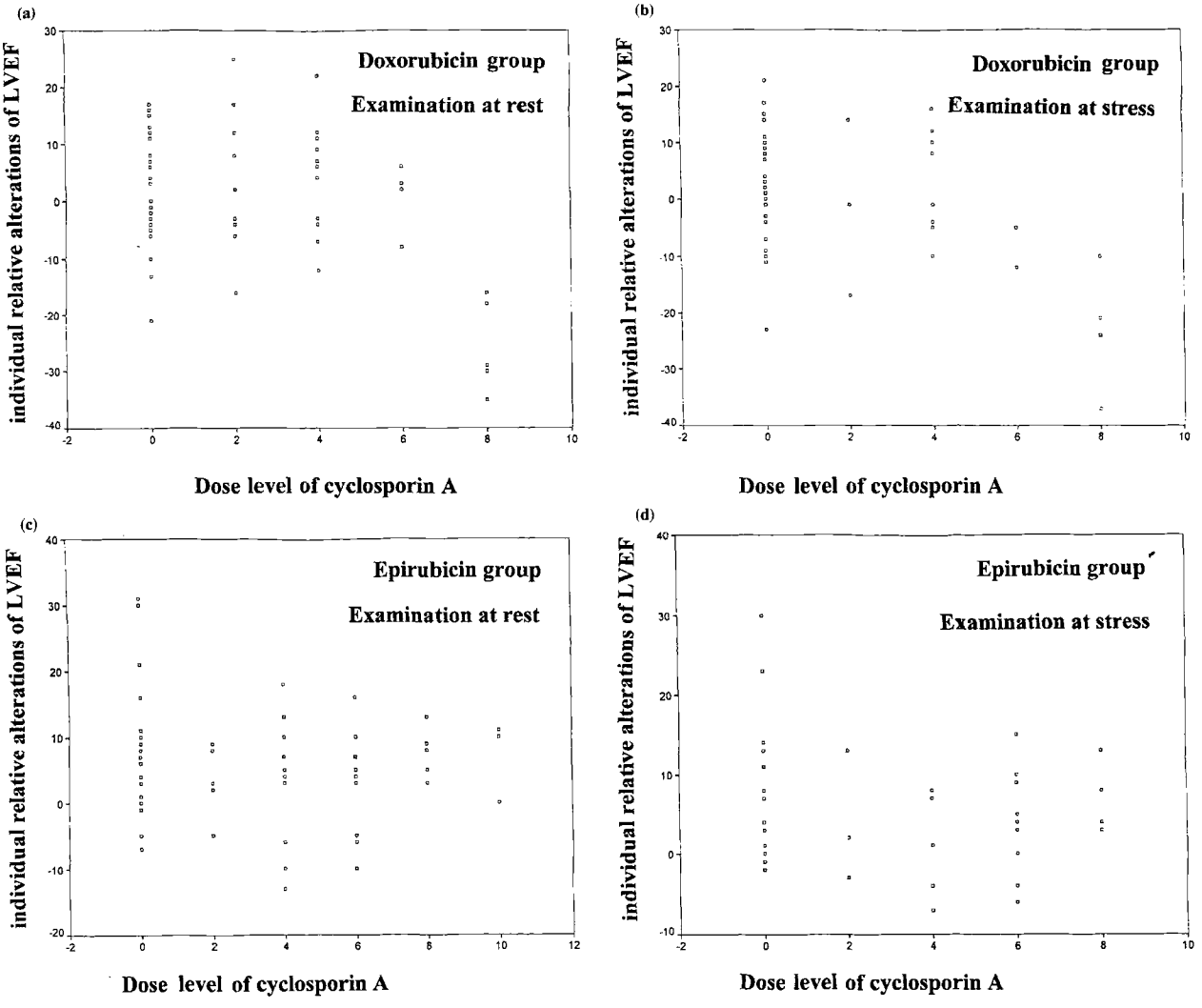

Fig. 1. Dependence of individual development of LVEF differences on chemotherapy with doxorubicin and epirubicin, especially combined with cyclosporin A compared to the start point. a) Dependence of LVEF at rest on dose levels of cyclosporin A in patients receiving doxorubicin. b) Dependence of LVEF at stress on dose levels of cyclosporin A in patients receiving doxorubicin. c) Dependence of LVEF at rest on dose levels of cyclosporin A in patients receiving epirubicin. d) Dependence of LVEF at stress on dose levels of cyclosporin A in patients receiving epirubicin.

tients, but belonged in principle to the schedule of chemotherapy used for patients having soft tissue sarcomas. This schedule consisted of up to 3 initial courses of high-dose ifosfamide (15.4 g/sqm) over 6 days, 3–4 weeks. If the tumor mass could be reduced only by 25% or less, up to 3 courses with anthracyclines (either 80 mg/sqm doxorubicin or 120 mg/sqm epirubicin as 30 min i.v. infusion) were applied, 3–4 weeks. If a sufficient tumor response was furthermore missing, 3 additional courses with anthracyclines and cyclosporin A were applied. Cyclosporin A was administered in a dose escalation schedule starting with dose level 2: loading dose of 2 mg/kg/2 h i.v. before the anthracycline followed by 6 mg/kg/24 h for 2 days continuous i.v. infusion immediately thereafter and increased during the following treatment courses step by step via 4 mg/kg/2 h and 12 mg/kg/24 h (dose level 4), 6 mg/kg/2 h and 18 mg/kg/24 h (dose level 6) up to 8 mg/kg/2 h and 24 mg/kg/24 h (dose level 8).

The total dose of doxorubicin administered in every course was (mean/SD) 141.8/29.0 mg, the total dose of epirubicin 208.1/22.8 mg. In the doxorubicin group, 6 197/3 296.4 mg cyclosporin A was administered and 6 316/3 009 mg in the epirubicin group. Nine patients received a prior treatment with doxorubicin (total dose: 297.8/126.4 mg) and 10 patients were treated with epirubicin (total dose: 390.5/227.6 mg) before the first administration of cyclosporin A, which causes the spread of the LVEF values at the first measurement in Fig. 1. A maximum of 10 courses of chemotherapy was performed. The total administered doses of doxorubicin amounted to 693.3/183.1 mg, and those of epirubicin to 1 040.5/392.7 mg.

Radionuclide ventriculography (RNV). The impact of the chemotherapeutic agents on the cardiac function was measured as a decrease of left ventricular ejection fractions (LVEF) using the RNV at rest and at stress (bicycle ergometry).

The 43 patients underwent 177 RNV examinations at rest. Stress examinations with bicycle ergometry were possible in 133 RNV.

In the doxorubicin group, 93/177 RNV examinations were performed and 84/177 ventriculographies in the epirubicin group. The RNV was performed immediately before the chemotherapy courses (doxorubicin group (mean/SD): 1.6 d/1.37 d, epirubicin group: 1.7 d/1.49 d) and 3–4 weeks after the last course (doxorubicin group: 24.3 d/3.27 d, epirubicin group: 25.3 d/4.18 d).

The examinations were performed before and during chemotherapy with both RNV studies applied up to 11 times in the follow-up of the patients (number of 1st examinations /.../; number of 11th examinations = 43/36/28/22/16/14/9/4/3/1/1).

The RNV was performed using the so-called multigated acquisition technique (MUGA):

- Thirty minutes before intravenous injection of 700 MBq Tc-99m-Pertechnetate, the patients received stannous pyrophosphate solution (TechneScan®-PYP) to achieve the optimum tin serum level of 0.197 mg/l for RBC labeling. Blockade of the thyroid was performed with 50 drops of Irenat®-solution (in about 100 ml mineral water) according to 1.0 g sodium perchlorate.
- The acquisition of gamma camera images was started 10 min after intravenous injection of the tracer.

The following three studies were acquired:

1. Rest study over 600 heart beats/left anterior oblique (LAO) projection.
2. Stress study over 400 heart beats/left anterior oblique (LAO) projection, if patients are able to undergo bicycle ergometry.
3. Rest study over 600 heart beats/anterior projection.

With respect to the reduced clinical status of many patients, bicycle ergometry was applied at a moderate intensity in order to increase heart frequency by at least 30% (during the whole time period of stress RNV study acquisition) as compared to heart frequency of rest study.

A Siemens SLC Orbiter® camera with LEAP collimator and ECG triggering (and monitoring) was used in all patients to acquire the studies mentioned above in planar technique. The Siemens MaxDelta® software (Version 7.2) running under the VMS® operating system (V. 5.5) of Digital Equipment (VAX® mainframe) was used for multigated data acquisition and processing.

The left ventricular ejection fraction was determined by the so-called 'manual' method: the contours of the left ventricle at the end-systolic and end-diastolic time were drawn by hand (joystick) as a separate region-of-interest (ROI) near to the ventricle as background region.

The LVEF was calculated on both LAO studies together with Fourier phase and amplitude analysis. The study in anterior projection was evaluated in order to calculate pictures of stroke counts, paradox motility and ejection fraction.

Statistics. In addition to the usual descriptive statistics of the acquired variables on LVEF, heart rate, age, etc., statistical correlations were performed using the Spearman rank correlation coefficient. The impact of the dosage of cyclosporin A, the chemotherapeutic agent (doxorubicin or epirubicin), and the age on individual LVEF development were evaluated by multivariate analysis of regression.

The SPSS® program (for Windows95®) was used as evaluation software.

RESULTS

The patients in the doxorubicin group were nearly 8 years older than the patients in the epirubicin group (mean age: 52 yrs vs. 44 yrs). The LVEF showed an average increase from 58.9% at rest to 67.0% at stress in the the doxorubicin group and 59.3% to 68.9% in the epirubicin group. The corresponding values for the heart rates at 90.0/115.7 min^{-1} and 92.3/117.3 min^{-1} respectively showed a percentage increase of 29% and 27% respectively, indicating a slight but sufficient ergometric stress near the desired value of 30% (over a time interval of 400 heart beats).

The univariate Spearman correlation between the dosage of cyclosporin A and the LVEF at rest/stress reveals a significant correlation only of the LVEF at stress in the doxorubicin group (Table 1), which corresponds to the decrease of LVEF in the dose step 8 in Fig. 1a and b.

To distinguish between the influence of cyclosporin A and the anthracycline, a multivariate analysis of regression was performed (Table 2). Because of its influence on both cardiotoxicity and LVEF (4), age was included in the analysis.

Significant effects could be observed in the doxorubicin group only: the patient's age had a negative influence on the individual LVEF development (at rest and at stress), whereas the negative effect of doxorubicin was seen in the at rest examination only. In contrast, the cumulative dose of cyclosporin A had a positive ($\beta > 0$) impact on the LVEF at rest and stress.

DISCUSSION

Anthracyclines are established chemotherapeutic drugs with a high antitumor potential. The underlying biochemical pathways of daunomycin and doxorubicin were described in 1977 by Goodman et al. (5).

Unfortunately, their use may be accompanied by severe cardiotoxic side effects (6–9). Cumulative doses of doxoru-

Table 1

Correlations

Spearman univariate correlation (SCC = Spearman correlation coefficient) Variable A/	Doxorubicin group			Epirubicin group		
Variable B	No. of exam.	SCC	p	No of exam.	SCC	p
EFREDIF/DOSE	93	−0.0778	0.459	84	0.1998	0.068
EFSTDIF/DOSE	**75**	**−0.2953**	**0.010**	50	0.1601	0.267
EFREST/DOSE	93	−0.1376	0.189	84	−0.1369	0.214
EFSTRES/DOSE	81	−0.0710	0.529	52	0.0184	0.897

At $p < 0.05$ significant SCC exceeding $|0.2|$ are plotted in bold.
Abbreviations: EFREDIF = Individual development of LVEF at rest (compared to first examination), EFSTDIF = Individual development of LVEF at stress (compared to first examination), EFREST = LVEF at rest, EFSTRESS = LVEF at stress, DOSE = dose level of cyclosporin A (accompaning anthracycline dosage).

bicin exceeding 550 mg/sqm can lead to congestive heart failure. Later, epirubicin was developed and found to possess about the same antitumor potential but only half the cardiotoxicity of doxorubicin.

In recent years, several attempts have been made to reduce the cardiotoxicity such as by encapsulation of doxorubicin in liposomes (10). Another clinical approach is the coadministration of the iron ion chelating agent dexrazoxane (ICRF-187).

According to pharmacological investigations, cardiac damage caused by anthracyclines better correlates with their peak plasma concentration than with their area under the concentration time curve (AUC) in plasma. Thus, a simple and effective clinical way to reduce anthracycline cardiotoxicity is their application by short time i.v. infusion instead of bolus injection (11–14).

The extent of cardiotoxic damage can be determined most accurately by invasive endomyocardial biopsy (15), but this method is not suitable for routine clinical pratice. The decrease of electrocardiographic voltage occurs too late for an early predictive monitoring of the patients (15). Ultrasonographic assessment is only worthwhile if frequent measurements are obtained (15), and magnetic resonance techniques are too expensive for use in routine clinical follow-up monitoring.

Therefore, the radionuclide ventriculography (RNV) remains the primary method of choice for this indication (16–18) and can help to avoid severe cardiotoxic side effects in high-risk patients (19). High-risk patients can be identified by at least one of the following criteria (19):

- Decline of $\geq 10\%$ in left ventricular ejection fraction from a normal baseline to $\leq 50\%$.
- Cumulative doxorubicin dose > 450 mg/sqm.
- Abnormal baseline of left ventricular ejection fraction of $< 50\%$.

The performance of RNV at stress can enhance the sensitivity of this examination in detecting high-risk patients (7, 20). Parmentier et al. (21) reported that the diastolic function is deteriorated earlier by the anthracyclines than the systolic function of RNV. Sufficient labeling yields of erythrocyte labeling with Tc-99m are necessary for a good contrast of the ventricular space. But the labeling yields are dependent on the blood concentration of anthracyclines (22) which can cause the deterioration of image evaluability in some patients. This problem can be solved by performing an in vitro labeling of the erythrocytes instead of an in vivo labeling.

Despite the different mean ages of the patients in both groups in this study, comparable results for the LVEF with a significant increase at stress could be stated. The univariate correlation and the corresponding plot revealed a slightly negative impact of the cyclosporin A doses on the LVEF at rest in the doxorubicin group. Naturally, these LVEF data are suspected to be influenced by the administration of doxorubicin. This fact made a multivariate exploration of the data necessary. In contrast to the previous results, the administration of cyclosporin A seems to have a positive impact on the individual development of LVEF. As expected, negative influences of age and doxorubicin on the LVEF could be confirmed. Therefore, the supposed slighly negative impact on the LVEF at rest in the univariate analyses must be seen as a sum effect of both cyclosporin A and doxorubicin (with a greater influence of doxorubicin). The positive impact of cyclosporin A on the LVEFs indicated that its reported cardiotoxicity-reducing effect of inhibition of the doxorubicin-induced mitochondrial calcium release (1, 23) is stronger than its opposite cardiotoxic effect of calcification and fibrosis (3) in clinical practice. It should be mentioned here again that the latter two effects have only been observed in mice when very high doses are used. If our data are interpreted very cautiously, at least a negative impact of cyclosporin A on the LVEFs could be excluded.

In the epirubicin group, no significant effects of age, cyclosporin A, and the epirubicin itself could be stated. In our opinion, the lower cardiotoxicity of epirubicin partly

Table 2

Results of analysis of multiple regression

	Difference in LVEF					
	Exam. at rest			Exam. at stress		
	β	SE(β)	p	β	SE(β)	p
Doxorubicin group						
Doxorubicin (cum.)	−0.520	0.009	0.040	−0.276	0.011	0.224
Cyclosporin A (cum.)	0.624	0.001	0.015	0.600	0.001	0.015
Age	−0.430	0.212	0.005	−0.366	0.279	0.040
Epirubicin group						
Epirubicin (cum.)	−0.473	0.005	0.070	−0.695	0.007	0.181
Cyclosporin A (cum.)	0.490	0.001	0.061	0.470	0.001	0.364
Age	0.267	0.102	0.068	−0.237	0.096	0.258

Abbreviations: cum. = cumulative dose, β = coefficient of regression, SE(β) = standard error of β.

compensates for the higher doses (comparing with doxorubicin), so that a negative impact on the LVEF could not be stated in a significant intensity (Table 2).

The finding that cyclosporine A does not increase the cardiotoxicity of high-dose anthracycline chemotherapy is of high significance for further clinical MDR modulation trials with respect to the influence of cyclosporine A and other MDR modulators on the pharmacokinetics of MDR-dependent cytostatics. The inhibition of the biliary excretion caused by cyclosporine A (24) leads to a higher retention with an increase of the AUC in plasma which could be demonstrated for the podophyllotoxin etoposide (25) as well as for the anthracyclines (26, 27) thus producing a significantly higher myelosuppression (28). As the same effect of cyclosporine A on the pharmacokinetics of the anthracyclines was found in the patients of this study (29) it can be concluded that the retention of the anthracyclines caused by cyclosporine A does not cause a higher cardiotoxicity rate.

CONCLUSION

This study indicates that the MDR modulator cyclosporin A has no negative impact on the individual development of LVEF at either rest or stress measured by RNV in clinical practice. Therefore, the advantage of using cyclosporin A to reduce MDR is not combined with an increase in cardiotoxicity.

Nevertheless, a cardiac monitoring, especially of those patients receiving high doses of anthracyclines, seems to be mandatory during chemotherapeutic treatment and must be performed in further clinical MDR modulation trials.

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