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Cardiac Troponin T and Creatine Kinase MB Mass Concentrations in Children Receiving Anthracycline Chemotherapy

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Anthracyclines (doxorubicin, daunorubicin, and derivatives) are among the most effective antineoplastic drugs for pediatric cancer with dose-limiting acute and long-term cardiotoxicity. The exact mechanism of the development of cardiomyopathy is still not clear. Anthracyclines may induce subclinical acute myocardial injury leading to lysis of a limited number of myocytes. Alternatively, myocytes may experience a transient loss of cytoplasmic membrane integrity. Both conditions may lead to a transient efflux of small amounts of cytoplasmic enzymes and other proteins specific to the heart muscle fibers.

To test these hypotheses we assayed plasma creatine kinase (CK) MB mass and cardiac specific troponin T (TnT). CKMB may be released even in case of reversible cell membrane injury, while prolonged elevation of TnT is the most sensitive and specific marker of limited myocardial necrosis.

Thirty-five anthracycline-containing chemotherapy courses in 22 children with cancer were analyzed. CKMB mass and TnT concentrations were within the normal range in all children before anthracycline therapy. Within 72 hours from anthracycline therapy no increment of one of these two marker proteins was detected (ANOVA for repeated measures, $P = 0.94$ [TnT] and 0.25 [CKMB]).

We conclude that only minimal if any acute necroses of cardiac myocytes occur after anthracycline therapy. Even membrane integrity appears to be maintained within the first 3 days after anthracycline therapy, in the absence of electrocardiographic or echocardiographic signs of acute cardiotoxicity.

Key words: troponin T, creatine kinase MB, anthracyclines, adverse effects, cardiotoxicity

INTRODUCTION

Anthracyclines (doxorubicin, daunorubicin, and derivatives) are among the most effective antineoplastic drugs for pediatric cancer [1–3]. Dose-limiting acute and long-term cardiotoxicity has been recognized more than 20 years ago [4–10]. The incidence of severe cardiotoxicity is dose-related [4], the susceptibility appears to be age-related, young children being at higher risk [9–13]. Recently it has been demonstrated that even very low cumulative doses of anthracyclines may cause permanent myocardial injury in a substantial fraction of young adults cured from cancer during childhood [12]. The extent of the myocardial lesion tends to progress during the years following the last administration of anthracycline therapy [12]. In a small proportion of anthracycline-treated patients, fatal congestive heart failure occurs months to years after the last antineoplastic treatment. Rarely the patients may experience an acute pericarditis-myocarditis syndrome [7,8]. Some patients succumb to sudden unexplained deaths [8]. The exact mechanism of the development of cardiomyopathy in anthracycline treated cancer patients is still not clear [14,15].

Acute myocardial injury can be sensitively and accurately identified by measurement of plasma concentrations to creatine kinase MB (CKMB) mass and cardiac specific troponin T (TnT), increases of these marker proteins being observed within 3 days from injury [16,17]. CKMB is a predominately cytoplasmic enzyme which may even be released in case of reversible cell membrane injury [18]. TnT is part of the tropomyosin complex. It is compartmentalized into a minor cytosolic and a major

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myofibrillary bound fraction. Prolonged release of cardiac TnT into plasma is generally assumed to reflect myocardial necrosis [19]. Recently troponin T has turned out to be the most sensitive and specific marker of limited myocardial necrosis. Soon after the injury TnT can be detected in the circulation [20–24]. In an animal study anthracycline administration was associated with TnT release to the circulation and the elevated TnT levels were correlated with clinical toxicity [25].

We report the results of a prospective study of TnT and CKMB mass plasma concentrations in children treated with anthracyclines for cancer.

PATIENTS AND METHODS

Patients

Within a 12 months period 35 courses of anthracycline therapy in 22 children (10 males and 12 females, aged 1 to 17 years, mean 6.65 years) were studied. Anthracycline chemotherapy was part of polychemotherapy protocols with either adriamycin (27 courses), daunorubicin (six courses), or idarubicin (two courses), it was administered intravenously through a central venous catheter system. In some of the patients a second (n = 5) or third (n = 4) anthracycline course was studied.

Eleven patients received anthracyclines for the first time. In the remaining 24 episodes the median cumulative anthracycline pretreatment dose was 180 mg/m^2 (range 60 to 460 mg/m^2).

Ten children were treated for acute leukemia or lymphoma, 12 for various solid tumors. Anthracyclines were used in varying combinations with other antineoplastic drugs. The anthracyclines were given intravenously as short infusions over 30 to 60 minutes via central venous catheters in most patients. Leukemia protocols included daunorubicin or doxorubicin 30 mg/m^2 weekly while solid tumor protocols applied doxorubicin 45 to 75 mg/m^2 within 3 days every 6 weeks. Idarubicin 36 mg/m^2 within 3 days was studied in two leukemia patients.

Cardiologic evaluation for signs of anthracycline toxicity was performed before each anthracycline course and 3 weeks thereafter. Standard toxicity criteria included repolarization disturbances on electrocardiogram and reduction of left ventricular fractional shortening on M-mode echocardiography [26].

Blood Collection

Two mL of venous blood was collected in EDTA-coated tubes before start of anthracycline therapy (timepoint zero), and 6, 12, 24, 48, and 72 hours thereafter in the first 17 courses studied. During the remaining 18 episodes samples were taken at time points 0, 24, 48, and 72 hours only.

Assays for Troponin T and CKMB Mass

Troponin T was measured using an enzyme immunoassay highly specific for cardiac TnT (Boehringer Mannheim, Mannheim, Germany). In adults, the upper limit of the reference interval is 0.20 μg/L [27]. Before the onset of the present study, this discriminator value had been confirmed in a healthy pediatric control population. In 30 children (17 males and 13 females, aged 1 to 16 years, mean 7.7 years) who were seen for preoperative examinations prior to minor surgery a 95th percentile of 0.14 μg/L was found.

CKMB mass concentrations were determined using an enzyme immunoassay (Abbott, Chicago, IL). The upper limit of the reference interval is 5 μg/L [28,29].

Control sera (high, low) from the assays' manufacturers were used for quality control (internal standards).

Statistics

The aim of the study was to detect at least an increment of 50% from baseline of either marker protein at a significance level of 0.05 with a power of 0.80 ($\beta = 0.20$). Based on the results in healthy controls for CKMB mass (mean 2.6 μg/L, SD 1.0) and TnT (mean 0.05 μg/L, SD 0.03) a sample size of 10 patients for CKMB analysis and 20 patients for TnT analysis was calculated.

Differences over time were analyzed by ANOVA with repeated measures. Differences of echocardiographic left ventricular shortening fractions before each anthracycline course and thereafter were analyzed by the paired T-test. To compensate biases by repeated analyses of the same individuals mean values of TnT and CKMB mass concentrations at each time point were calculated and used for ANOVA in the eight children with two or three anthracycline courses studied. Each patient was entered only once into ANOVA procedures.

There were no missing values for TnT. Eight missing values in the CKMB mass series were replaced by the mean value at the respective time point in the remaining patients to allow analysis by the ANOVA procedure.

RESULTS

At baseline no child showed clinical, electrocardiographic, or echocardiographic signs of myocardial injury. No child showed increments of either CKMB or TnT 6 and 12 hours, and 1, 2, or 3 days after anthracycline therapy, respectively (ANOVA for repeated measures, $P = 0.94$ [TnT] and 0.25 [CKMB], respectively; Table I, Figs. 1, 2; data for 6 and 12 hours not shown).

Upon cardiologic short-term follow-up, clinical signs of acute or subacute anthracycline toxicity were not observed and myocardial injury was undetectable by noninvasive methods (ECG and M-mode echocardiography: shortening fraction before and 3 weeks after anthracy-

TABLE I. Plasma Concentrations (Median, 25th/75th Percentiles, Means ± SD, Range) of CK-MB Mass and TnT, Respectively, in Pediatric Cancer Patients Before (Day 0) and on Day 1, 2, and 3 Following Anthracycline Therapy

	Time point	Median (25th/75th percentiles)	Mean (± SD)
CKMB mass (μg/L)	day 0	2.0 (1.3/2.6)	2.0 (± 1.2)
	day 1	2.1 (1.3/2.6)	2.1 (± 1.0)
	day 2	1.8 (1.1/2.4)	1.8 (± 1.0)
	day 3	2.0 (1.4/2.1)	2.0 (± 1.2)
TnT (μg/L)	day 0	0.05 (0.04/0.08)	0.07 (± 0.04)
	day 1	0.05 (0.04/0.09)	0.06 (± 0.03)
	day 2	0.04 (0.04/0.09)	0.06 (± 0.03)
	day 3	0.04 (0.04/0.09)	0.07 (± 0.04)

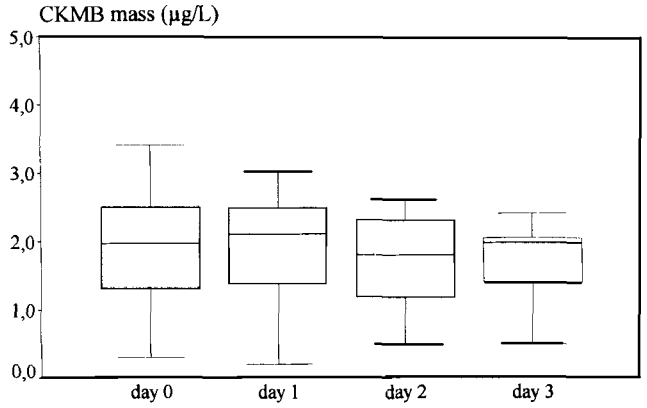

Fig. 1. Unchanged plasma levels of creatine kinase (CK) MB mass before anthracycline therapy (day 0) and 1, 2, and 3 days thereafter; ANOVA for repeated measures, $P = 0.25$ [upper limit of normal 5.0 μg/L, box-and-whiskers plots demonstrating the median value (horizontal line), the interquartile range (box), and the range between minimum and maximum value (whiskers)].

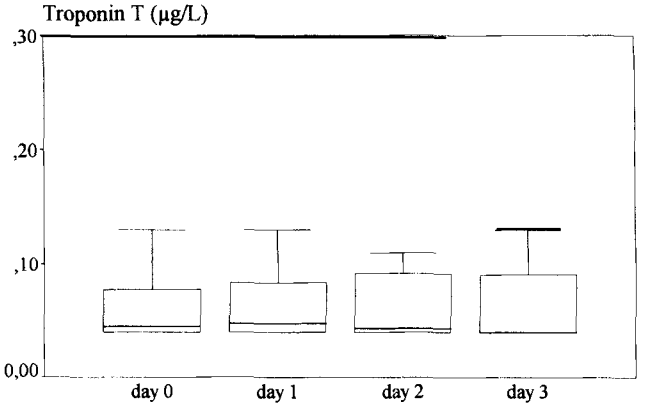

Fig. 2. Unchanged plasma levels of troponin T (TnT) before anthracycline therapy (day 0) and 1, 2, and 3 days thereafter; ANOVA for repeated measures, $P = 0.94$ (upper limit of normal 0.20 μg/L, box-and-whiskers plots, for explanation see Figure 1). On day 3 more than 50% of patients had values below the detection limit of the TnT assay (0.04 μg/L). Therefore the median value, the 25th percentile, and the minimum value were identical (0.04 μg/L). (On the other days the 25th percentiles and the minimum values were identical.)

cline treatment [mean ± SD] 31.7 [±3.2] and 31.4% [±1.8], respectively, P = n.s.).

DISCUSSION

Among other lesions several postmortem and endomyocardial biopsy studies have documented striking loss of and degeneration of the remaining cardiomyocytes [30–33]. Thus anthracyclines may induce subclinical acute myocardial injury leading to lysis of a limited number of myocytes. Alternatively, myocytes may experience a transient loss of cytoplasmic membrane integrity. Both conditions may lead to a transient efflux of small amounts of cytoplasmic enzymes and other proteins specific to the heart muscle fibers. Interstitial fibrosis is also a feature of anthracycline cardiotoxicity [31–34].

TnT is highly sensitive to detect myocardial necroses, e.g., in adults after extraordinary endurance exercise, or in patients with unstable angina pectoris [21,24]. TnT release is observed within the first 24 hours after limited myocardial injury, e.g., after myocardial infarction with early reperfusion. TnT stays increased for several days [19]. In an animal study anthracycline administration was associated with TnT release to the circulation and the elevated TnT levels were correlated with clinical toxicity [25]. Elevated CKMB mass is the earliest detectable plasma marker of myocardial infarction. It peaks 12 to 24 hours after the onset of chest pain and returns to normal after 3 days [16]. In a previous study on side branch-occlusion during percutaneous transluminal coronary angioplasty which causes limited myocardial injury, CKMB mass release peaked at 24 hours [23].

Despite this high sensitivity of TnT and the specificity of both tests, we were unable to detect elevated CKMB or TnT concentrations in pediatric cancer patients within the first 3 days after the start of an uncomplicated chemotherapy course including anthracyclines. Apparently the extent of myocardial injury in these patients is below the detection limit of the assay.

However, alternative potential mechanisms of anthracycline cardiomyopathy leading to injury of the intersti-

tium or autoimmune mechanisms cannot be detected by acute CKMB or TnT release. Such lesions have been described by several authors. Caulfield and Bittner demonstrated that a single injection of adriamycin caused permanent alteration of the myocardial interstitium in the rat [34]. Huber described the generation of cytolytic T lymphocytes in response to anthracycline treated myocardial cells, suggesting a contribution of autoimmune mechanisms in anthracycline cardiotoxicity [35].

We conclude that only minimal, if any, acute necrosis of cardial myocytes occur after uncomplicated anthracycline therapy. Even membrane integrity appears to be maintained within the first 3 days after anthracycline therapy, in the absence of electrocardiographic or echocardiographic signs of acute cardiotoxicity.

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