CHRONIC ANTHRACYCLINE CARDIOTOXICITY: MOLECULAR AND FUNCTIONAL ALTERATIONS IN THE POST-TREATMENT FOLLOW UP

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Introduction

Anthracycline antibiotics (ANT) represent one of the most effective and frequently used anticancer drugs ever developed. [1] However, their clinical use is considerably hampered by a risk of cardiac toxicity, which can seriously affect the long-term morbidity, mortality and quality of life of cancer survivors. Among various forms of ANT cardiotoxicity, the major clinical concerns are related to the chronic ones, which are associated with development of cardiomyopathy and heart failure. These particular forms of ANT cardiotoxicity stay clinically silent during the anticancer treatment, while they could manifest themselves months or even years after the completion of the therapy (early and late onset form, respectively). [2] Indeed, it is very difficult to directly assess ANT-induced myocardial changes and their further development in the post-treatment period in clinical settings. However, there is also a surprising lack of experimental data on the post-exposure follow up of chronic anthracycline cardiotoxicity from animal models. Despite endless controversy concerning precise mechanism of ANT cardiotoxicity, the majority of authors suppose oxidative stress to be the main cause of ANT-induced myocardial injury. [3] Furthermore, recent data suggest that mitochondria can be the main target of ANT toxicity. However, further development of these changes in the post-treatment period is unknown. In addition, virtually nothing is known about response of key protective pathways coordinating antioxidant response to oxidative stress (Nrf2 pathway) and mitochondrial biogenesis program counteracting mitochondrial deteriorations (PGC1α pathway).

Aims

The aim of this study was to analyze functional and molecular changes induced in rabbit myocardium by chronic ANT treatment and its progression in the post-treatment follow up with special focus on the Nrf2 and PGC1α pathways.

Methods

Chronic ANT cardiotoxicity was induced in rabbits using well established model [4] by administration of daunorubicin (DAU, 3 mg/kg, *i.v.*, weekly for 10 weeks, n=27). Control animals received saline in the same schedule (n=19). A week after the last dose, animals were randomized and a half of them was terminated, while the rest underwent post-treatment followed up for next 10 weeks (FU). Cardiac function and structure were examined by echocardiography (GE Vivid4, 10 MHz probe) and plasma concentrations of the cardiac troponin T (biomarker of cardiotoxicity) were analyzed using Elecsys Troponin T hs assay (Roche Diagnostics). Parameters of oxidative stress (MDA, GSSG, GSH) were analyzed using validated HPLC methods [5]. The expression of Nrf2 and PGC1α and their target genes were analyzed using qRT-PCR (7500HT Fast Real-Time PCR System, Applied Biosystems). Transcriptionally active form of Nrf2 was analyzed in nuclear fraction using commercial ELISA kit (TransAM Nrf2, Activemotif). Enzyme-coupled spectrophotometric assays for measurement of activity of citrate synthase, respiratory chain complex I, GPx and GR were performed as previously described [6, 7] and activity of GST was measured using SensoLyte fluorimetric kit (AnaSpec).

Results

Chronic DAU treatment induced significant decrease of the LV systolic function with further marked progression in the FU (to 47.6 % of the initial values, $p \leq 0.001$). In the latter period a LV diameter became significantly increased ($p \leq 0.01$). DAU administration also induced a progressive increase of cardiac troponin T in plasma and the concentrations remained elevated several weeks in the post-treatment FU. DAU-treatment resulted into the increase of the LV MDA content with further significant increase in the FU ($p \leq 0.01$). However, the MDA values were only

loosely associated with the parameters of cardiac toxicity (cardiac troponin T, LV FS, $R<0.45$). Furthermore, an increase of LV GSSG content was found, nevertheless, GSH increased in a similar manner and therefore, the key GSSG/GSH ratio remained unchanged in both study periods. Furthermore, we found no change in LV expression and nuclear transcriptional activity of a master regulator of the antioxidant response – Nrf2. No change due to DAU treatment was therefore also identified in important Nrf2 targets like CuZnSOD or enzymes involved in glutathione system maintenance: GCLC, GPx, GR and GST. On the other hand, significant ($p\leq0.001$), but Nrf2-independent regulation was detected in the case of expression of MnSOD, NQO1 and HO-1. While the former antioxidant molecules were markedly downregulated (by 47.2 % and 21.6 %), cytoprotective HO-1 was significantly induced due to the treatment (by 2.28 fold). These changes remained profound and significant ($p\leq0.01$) also in the FU. Moreover, there was relatively strong association of these changes with the toxicity parameters ($R\geq0.75$). Moreover, DAU-treatment induced significant damage to LV mitochondria (a decrease of complex I activity by 31.3 % and drop of the citrate synthase activity by 19.6 %, $p\leq0.05$). However, no induction of mitochondrial biogenesis pathway was found in response to the treatment-induced damage in both study periods. Instead, downregulation of the pathway master regulator (PGC1α) and its downstream pathway members (NRF1, TFAM) was observed, especially in the post-treatment FU. These findings corresponded with the ultimate decrease of expression of key mitochondrial proteins encoded by both nuclear and mitochondrial genome. With regards to the complex I, expression of nuclear-encoded subunit (NDUFS2) was significantly suppressed already at the end of treatment, while mitochondria-encoded one (ND1) was not significantly downregulated until the post-treatment period.

Discussion

Chronic ANT treatment induced LV dysfunction, which progressed further toward congestive heart failure and LV dilation in the post-treatment follow up. Plasma cardiac troponin T measurements are indicating that cardiomyocyte degeneration and non-programmed cell death increase with cumulative dose and importantly that ongoing damage takes place many weeks after the end of the ANT treatment. Although myocardial lipoperoxidation increased significantly even in the FU, relatively poor association with the cardiotoxicity parameters argues against oxidative stress as the main executive factor of the cardiotoxicity. Furthermore, unchanged GSSG/GSH ratio suggests that glutathione system may be relatively competent despite the ANT-induced cardiac damage. Of note, we have for the first time shown that Nrf2 pathway controlling antioxidant response is not induced by chronic ANT treatment either at the end of treatment or in the drug free follow up period. Although majority of Nrf2 target genes showed no change due to the ANT treatment, important antioxidant enzymes (MnSOD and NQO1) were downregulated, while HO-1 was markedly induced. All these changes persisted in the FU and they deserve further investigation as they were relatively strongly associated with the cardiotoxicity. Of note, although we have found evidence for ANT-induced mitochondrial damage (including complex I impairment), no induction of mitochondria biogenesis pathway, which may provide compensation for these alterations, was revealed. Instead, we found that this pathway is downregulated, especially in the FU and this may be connected with progression of the mitochondrial damage and transition of LV dysfunction to heart failure.

Conclusions/Summary

In the present study, we describe progression of chronic anthracycline cardiotoxicity in the post-treatment follow up. Our data shows that ANT-induced global oxidative stress need not be the direct executive factor responsible for progression of anthracycline cardiotoxicity and development of heart failure. We also demonstrate that Nrf2 pathway providing antioxidant response is not induced by anthracycline treatment. Nrf2-independent regulation of expression of MnSOD, NQO1 and HO-1 deserves further study as it may provide new explanation for development of ANT cardiotoxicity. Furthermore, despite evident damage to mitochondria which persisted or developed further in the follow up, no induction of mitochondrial biogenesis pathway controlled by PGC1α was found. Instead, the pathway was suppressed, particularly in the post treatment period and this may be connected with ongoing myocardial damage and transition to heart failure.

References

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(*upraveno pro potřeby vzoru)*