Basic Science and Experimental Studies

Ischemic Cardiomyopathy Affects the Thioredoxin System in the Human Myocardium

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ABSTRACT

Background: Oxidative stress due to reactive oxygen species (ROS) production is a key factor in the development of heart failure (HF). This study investigated the thioredoxin (Trx) system, which plays a major role in antioxidant defense, in patients suffering from ischemic (ICM) or dilated (DCM) cardiomyopathy.

Methods and Results: Myocardial tissue from ICM (n = 13) and DCM (n = 13) patients, as well as septal tissue of patients with aortic stenosis but without diagnosed hypertrophic cardiomyopathy or subaortic stenosis (control; n = 12), was analyzed for Trx1, Trx-interacting protein (TXNIP) and E3 ligase ITCH (E3 ubiquitin-protein ligase Itchy homolog) expression. Trx-reductase 1 (TXNRD1) amount and activity, cytosolic cytochrome C content, and apoptosis markers were quantified by means of enzyme-linked immunosorbent assay and multiplexing. Compared with control samples, ITCH and Trx1 expression, TXNRD1 amount and activity were reduced and TXNIP expression was increased in ICM (ITCH: $P = .013$; Trx1: $P = .028$; TXNRD1 amount: $P = .035$; TXNRD1 activity: $P = .005$; TXNIP: $P = .014$) but not in DCM samples. A higher level of the downstream apoptosis marker caspase-9 (ICM: 582 ± 262 MFI [$P = .995$]; DCM: 1251 ± 548 MFI [$P = .002$], control: 561 ± 214 MFI) was detected in DCM tissue. A higher expression of Bcl-2 was found in DCM ($P = .011$).

Conclusion: The Trx system was impaired in ICM but not in DCM. ITCH appeared to be responsible for the down-regulation of the Trx system. ROS-induced mitochondrial instability appeared to play a role in DCM. (*J Cardiac Fail 2019;25:204–212*)

Key Words: Heart failure, thioredoxin, cardiomyopathy, ITCH, TXNIP, E3 ligases.

Heart failure (HF) remains one of the greatest health issues worldwide, with a prevalence of 1%–2%, a lifetime risk of 21%–30% by the age of 55 years, and a 5-year survival rate of 35%.[1–4] Understanding the mechanisms of HF is important for developing future therapy strategies. Oxidative stress due to excessive production of reactive oxygen species (ROS) relative to the antioxidant defense is thought to be a key factor in the development of HF.[5–7] The thioredoxin (Trx) system plays a major role in antioxidant defense along with the glutathione system.[8] The central molecule of the Trx system is Trx1 which contains a dithiol/disulfide motif within its redox active site, acting as an ROS scavenger. Despite this function, Trx1 serves as an electron donor for ROS scavengers such as peroxiredoxin.[9] In addition, Trx1 binds to apoptosis signal–regulating kinase 1 (ASK1), thus inhibiting ASK1.[10] To fulfill these tasks, Trx1 needs to persist in a reduced state that is mediated by Trx1 reductase (TXNRD1), using NADPH to transform Trx1 from an oxidized to a reduced state.[11] To regulate the Trx system, Trx-interacting protein (TXNIP) works as an endogenous inhibitor through binding Trx1's active site.[9]

The Trx system is regulated by proteins that are connected to further cellular systems that in turn are involved in cardiac remodeling and HF.[12,13] For example, the E6-AP carboxyl terminus (HECT)–type E3 ligase ITCH (E3 ubiquitin-protein ligase Itchy homolog) controls the degree to which TXNIP is degraded by the proteasome via ubiquitination.[14]

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Within the ubiquitin-proteasome system (UPS), E3 ligases fulfill the task of connecting ubiquitin to the target protein, leading to protein degradation or activation of signaling pathways, depending on the type of ubiquitination.[15]

ITCH is one of the more than 600 known E3 ligases. ITCH possesses 4 domains: the HECT domain, a WW domain, a Ca^{2+}-dependent phospholipid-binding C2 domain, and a proline-rich motif.[16] While the WW domain identifies target proteins, the HECT domain connects ubiquitin to target proteins. It has been reported that mice overexpressing ITCH had decreased levels of TXNIP, higher levels of Trx1, and higher survival rates after cardiotoxic intervention (eg, myocardial infarction surgery or doxorubicin injection) compared with their wild-type littermates.[17]

Considering the results of Otaki et al[17] in a rodent model, we investigated human tissue from patients undergoing cardiac remodeling caused by cardiomyopathy related to impairment of the Trx system and ITCH expression. Our hypothesis included the assumption that UPS dysfunction was involved in the pathogenesis of HF, as has been shown in previous studies.[18–23] An impaired UPS includes altered expression of E3 ligases such as ITCH. Therefore, we postulated that (1) if ITCH expression were reduced in the myocardium from patients with dilative cardiomyopathy (DCM) or ischemic cardiomyopathy (ICM), then protein expression of TXNIP would increase; (2) if TXNIP expression were increased in DCM or ICM patients, then protein expression of Trx1 would decrease; and (3) if Trx1 expression were decreased in DCM or ICM patients, then apoptotic activity within the myocardium would be increased.

Methods

Sample Collection and Preparation

Collection of tissue took place at the Clinic for Cardiac Surgery at the Heart Center Leipzig, Germany. The study complied with the Declaration of Helsinki. Following approval by the local IRB committee of the medical faculty of the University of Leipzig (No. 240/16ek), patients gave written informed consent.

Human myocardial tissue was obtained from the cardiac apex during left ventricular assist device implantation. The control group consisted of septal tissue obtained during Morrow resection of patients undergoing surgical aortic valve replacement because of aortic stenosis without diagnosed heart failure, hypertrophic cardiomyopathy, or subaortic stenosis. This type of control tissue was chosen because the patient cohort did not suffer from heart failure nor were they diagnosed with ICM or DCM, and fresh native apex biopsies in amounts necessary for the protein analysis panel of this study are not available from healthy control subjects. Myocardial tissue from ICM (n = 13), DCM (n = 13) and control (n = 12) patients was prepared, dissected, and stored for protein and enzyme analysis at −80°C.

Western Blot

Frozen tissue samples were homogenized in lysis buffer (50 mmol/L Tris, 1 mmol/L EDTA, 150 mmol/L NaCl, 1% nonidet-P40, 0.25% deoxycholic acid sodium salt, 1× protease, phosphatase inhibitor cocktail (Thermo Scientific, Waltham, Massachusetts), and 1% phenylmethylsulfonyl fluoride [PMSF]), sonicated with the use of an ultrasonic homogenizer and centrifuged at 14,000 rpm for 5 minutes at 4°C. After protein extraction, the protein content was determined with the use of a bicinchoninic acid (BCA) assay for colorimetric quantification (Pierce BCA Protein Assay Kit; Thermo Fisher Scientific) by measuring the absorbance at 560 nm on a spectrophotometer. Western blot analyses were performed with the use of specific primary antibodies against ITCH (anti-ITCH/AIP4 antibody; Abcam, Cambridge, United Kingdom), TXNIP (Cell Signaling, Frankfurt, Germany), Trx1 (Cell Signaling), and glyceraldehyde-3-phospate dehydrogenase (Hytest, Shanghai, China). Each gel contained a balanced number of samples from all 3 groups. Horseradish peroxidase (HRP)−conjugated secondary antibodies (Sigma, St Louis, Missouri) were used for ITCH and Trx1 detection. For TXNIP detection, a biotin-conjugated secondary antibody (anti−rabbit biotin; Sigma) and a tertiary HRP-conjugated antibody (streptavidin-HRP antibody; Thermo Fisher Scientific) were used. Bands were visualized by initiation of chemiluminescence with the use of the Super Signal West Dura Extended Duration Substrate (Thermo Fisher Scientific). Densitometry was performed with the use of the Fusion Vision Capt v16 Software (VWR, Radnor, Pennsylvania).

Enzyme-Linked Immunosorbent Assay

Quantification of TXNRD1 activity was performed via the enzyme-linked immunosorbent assay (ELISA) Human Trx-Reductase 1 ELISA Kit (Abcam). All ELISAs were performed according to the manufacturer's instructions. ELISAs were analyzed with the use of the M1000 Pro Tecan microplate reader and I-control software (both from Tecan Group, Männedorf, Switzerland).

ROS-Producing Enzyme Activity

Frozen tissue samples were homogenized in lysis buffer, sonicated, and centrifuged at 16,000*g* at 4°C for 10 minutes. The activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Roth, Arlesheim, Switzerland), a potent reactive ROS-producing enzyme, was measured photometrically via an UV/Vis spectrometer (Lamda 20; Perkin Elmer, Rodgau, Germany) at 550 nm. After addition of 4 mmol/L cytochrome C and 100 μmol/L NADH to the tissue homogenates, the proportion of cytochrome C reduction in the presence NADH was determined.

Multiplex Analysis

For quantification of cytosolic cytochrome C and cleaved caspase-3 (cCasp3), a multiplex Apoptosis Human Panel Kit

(Thermo Fisher Scientific) was used on a Luminex 200 platform (Austin, Texas). In addition, caspase-8 and -9, c-Jun N-terminal kinase (JNK), B-cell lymphoma 2 (Bcl-2), Bcl-2−associated death promotor protein (Bad), protein kinase B (Akt), and the tumor suppressor gene p53 were quantified with the use of the 7-Plex Early Apoptosis Magnetic Bead Kit (Merck Millipore, Burlington, Massachusetts).

Cytosolic fractions of cardiac tissue were obtained by mincing 50 mg of frozen cardiac tissue with the use of a mortar and pestle chilled with liquid nitrogen. The tissue powder was immediately transferred into 500 μL lysis buffer (50 mmol/L Tris, 1 mmol/L EDTA, 150 mmol/L NaCl, 1% nonidet-P40, 0.25% deoxycholic acid sodium salt, 1× protease, phosphatase inhibitor cocktail (Thermo Scientific), and 1% PMSF). Samples were vortexed, incubated for 30 minutes on ice, and centrifuged at 10,000*g* for 10 minutes to separate the cytosolic fraction from nuclei and mitochondria. The supernates displayed the cytosolic fraction and were removed. The protein concentration of the supernate was determined as mentioned above (Western blot), and samples were used for multiplex analyses.

Statistical Analysis

Statistical analysis was performed with the use of SPSS 23.0 (IBM, Armonk, New York). Differences between the groups of metric variables were analyzed by means of 1-way analysis of variance, including the Levene test to determine the equality of variances. For equal variances, data were analyzed with the use of the Scheffe post hoc test. In case of variance inequality, the Welch test was carried out for global testing and the Dunnett T3 method was performed for the post hoc algorithms. Nominal variables were analyzed with the use of theFisher exact test. Unless stated otherwise, values represent mean ± SD. Statistical significance was defined as $P \leq .05$.

Results

Patient Characteristics

Group differences regarding sex, age, ejection fraction, coronary heart disease and myocardial infarction are presented in Table 1. The study groups were similar for body mass index, status of smoking, and comorbidities, such as hypertension and diabetes mellitus type II. Significant differences in age underscored the fact that DCM and ICM manifest earlier in life than aortic stenosis. In the control group, no HF with reduced ejection fraction occurred. The difference between groups regarding CHD is due to the fact that ICM is a subclass of CHD according to the International Statistical Classification of Diseases and Related Health Problems (ICD-10). No significant difference was found between the control and DCM groups regarding CHD. The higher rate of myocardial infarction in the ICM group resulted from the fact the CHD is a precondition of myocardial infarction and was more prevalent, therefore leading to a higher rate of myocardial infarction, in the ICM group.

The following results should be interpreted cautiously, because group differences were not adjusted for influencing factors such as age and sex.

ITCH, TXNIP, and Trx1 Expression

Western blot analysis revealed lower expression levels of ITCH and Trx1 in ICM (ITCH: 0.32 ± 0.12 arbitrary units [$P = .013$]; Trx1: 0.84 ± 0.34 [$P = .028$]) but not in DCM (ITCH: 0.66 ± 0.47 [$P = .939$]; Trx1: 1.05 ± 0.41 [$P = .206$]) compared with control (ITCH: 0.70 ± 0.23; Trx1: 1.36 ± 0.36; Fig. 1A and C). TXNIP expression was higher in ICM (1.86 ± 1.08; $P = .014$) and remained constant in DCM (1.01 ± 0.38; $P = .371$) compared with control (0.82 ± 0.22; Fig. 1B).

Trx1 System Activity

Reduced concentration and reduced activity of TXNRD1 was found in ICM (TXNRD1 concentration: 0.06 ± 0.02 ng/mL [$P = .035$]; TXNRD1 activity: 0.0005 ± 0.0003 arbitrary units [$P = .005$]) but not in DCM (TXNRD1 concentration: 0.07 ± 0.02 ng/mL p$P = .433$]; TXNRD1 activity: 0.0007 ± 0.0001 [$P = .120$]) compared with control (TXNRD1 concentration: 0.08 ± 0.02 ng/mL; TXNRD1 activity: 0.0009 ± 0.0002; Fig. 2).

Table 1. Patient Characteristics and Comorbidities

Characteristic	Control(n = 12)	DCM(n = 13)	ICM(n = 13)	*P* Value
Male sex, n (%)	6 (50%)	12 (92%)	13 (100%)	.002
Age, y	70.8 ± 8.0	63.0 ± 5.8	56.0 ± 9.5	<.001
Ejection fraction, %	59.0 ± 16.4	16.0 ± 4.2	20.0 ± 6.1	<.001
BMI, kg/m^2	28.4 ± 6.4	28.5 ± 6.4	28.0 ± 6.3	.983
Hypertension, n (%)	9 (75%)	8 (62%)	8 (61.5%)	.757
CHD, n (%)	5 (42%)	5 (39%)	13 (100%)	.001
DM type II, n (%)	1 (8%)	4 (31%)	4 (31%)	.357
MI, n (%)	1 (8%)	2 (15%)	8 (62%)	.011
Smoker, n (%)	1 (8%)	4 (31%)	6 (46%)	.139

BMI, body mass index; CHD, coronary heart disease; DCM, dilated cardiomyopathy; DM, diabetes mellitus; ICM, ischemic cardiomyopathy; MI, myocardial infarction.

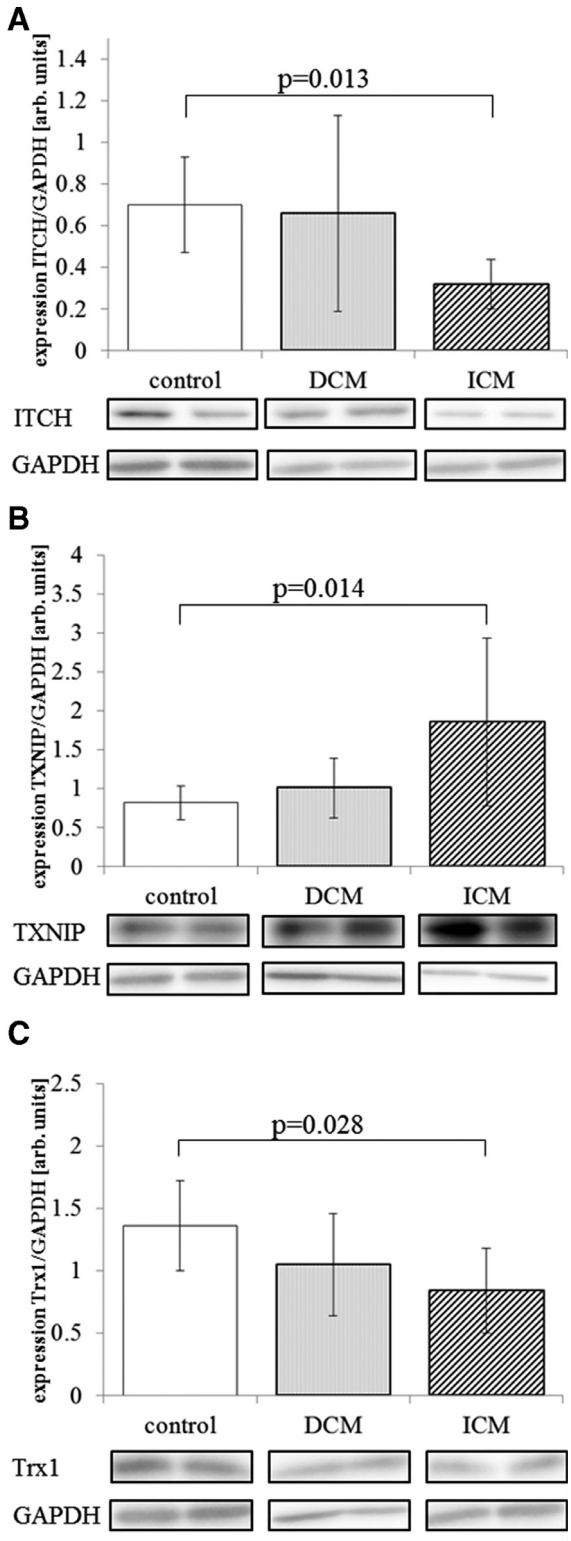

Fig. 1. Relative protein expression of (A) ITCH, (B) TXNIP, and (C) Trx1 in myocardial tissue from control (n = 12), DCM (n = 13), and ICM (n = 13) patients. DCM, dilated cardiomyopathy; GAPDH, glyceraldehyde-3-phospate dehydrogenase; ICM, ischemic cardiomyopathy; ITCH, E3 ubiquitin-protein ligase Itchy homolog; Trx1, thioredoxin 1; TXNIP, thioredoxin-interacting protein.

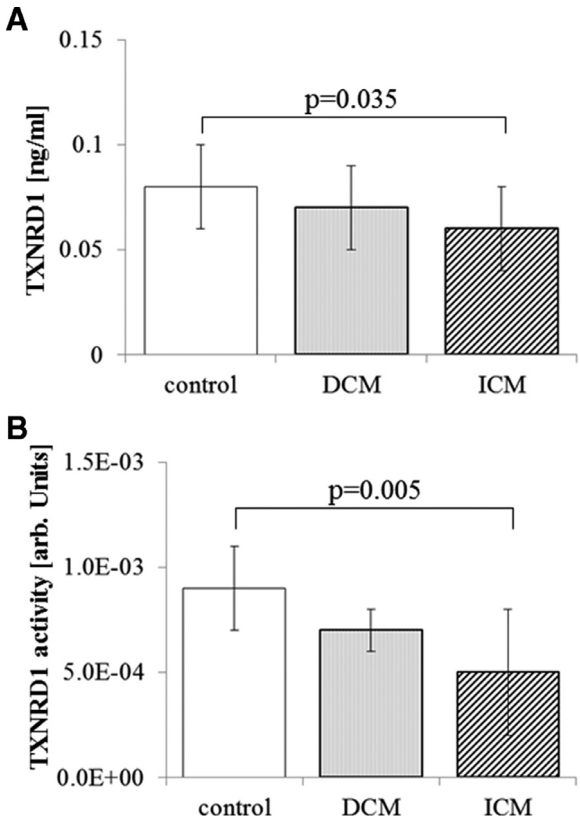

Fig. 2. (A) Expression oand (B) activity of TXNRD1 in myocardial tissue from control (n = 12), DCM (n = 13), and ICM (n = 13) patients. DCM, dilated cardiomyopathy; ICM, ischemic cardiomyopathy; TXNRD1, thioredoxin reductase 1.

NADPH-Oxidase Activity and Cytochrome C Release

NADPH-oxidase activity, a proxy for ROS production, significantly differed among the ICM (0.15 ± 0.03 mU/mg), DCM (0.35 ± 0.09 mU/mg), and control (0.29 ± 0.04 mU/mg; $P = .030$) groups, but post hoc analysis could not further confirm a significant result (Fig. 3A).

A significant difference for cytosolic cytochrome C was detected among the groups (ICM: 1476 ± 970 ng/mL; DCM: 2313 ± 1300 ng/mL, control: 1286 ± 667 ng/mL; $P = .042$), being highest in the DCM group, but post hoc tests did not detected a statistical difference ($P = .065$; Fig. 3B).

Induction of Apoptosis

Multiplex analysis showed that the apoptosis pathway upstream molecules p53 (ICM: 47.2 ± 16.6 MFI; DCM: 52.3 ± 18.2 MFI; control: 37.7 ± 10.7 MFI; $P = .088$; Fig. 4A) and Bad (ICM: 1302 ± 404 MFI; DCM: 1287 ± 291 MFI; control: 970 ± 375 MFI; $P = .055$; Fig. 4B) were similar among the groups. Quantification of Akt and JNK protein levels indicated no difference among ICM, DCM, and control groups (Akt: $P = .297$; JNK: $P = .140$; Fig. 4C, D).

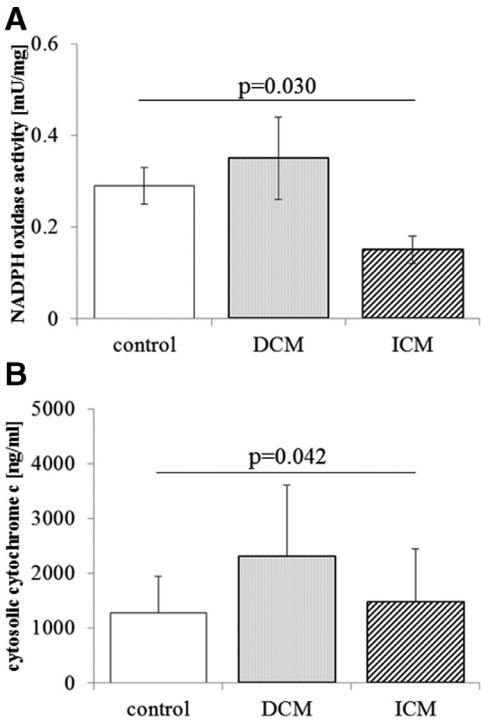

Fig. 3. (A) Activity of NADPH oxidase and (B) expression of cytosolic cytochrome C in myocardial tissue from control (n = 12), DCM (n = 13), and ICM (n = 13) patients. DCM, dilated cardiomyopathy; ICM, ischemic cardiomyopathy; NADPH, nicotinamide adenine dinucleotide phosphate.

Caspase-9, a downstream marker of the intrinsic apoptotic pathway, increased in DCM (1251 ± 548 MFI; $P = .002$) but not in ICM (582 ± 262 MFI; $P = .995$) compared with control (561 ± 214 MFI; Fig. 4G). However, cleaved caspase-3 expression was similar among ICM (1.2 ± 1.2 ng/mL), DCM (1.1 ± 0.7 ng/mL), and control (0.9 ± 0.6 ng/mL, $P = .741$; Fig. 4H) groups. Expression levels of caspase-8, the initiator caspase of the extrinsic apoptotic pathway, did not differ among the groups (ICM: 36.9 ± 5.4 MFI; DCM: 37.6 ± 5.5 MFI,-control: 39.6 ± 6.4 MFI; $P = .516$; Fig. 4F).

Bcl-2 Expression Analysis

Compared with control (20.7 ± 4.4 MFI), higher expression of Bcl-2 was found in DCM (27.3 ± 5.0 MFI; $P = .011$) but not in ICM (22.3 ± 5.5 MFI; $P = .729$; Fig. 4E).

Discussion

In this study, we investigated the Trx system in failing human hearts suffering from ICM or DCM and characterized its connection to the UPS and the induction of apoptosis pathways. Two major findings were determined: first, the Trx system was impaired in the myocardium of ICM patients; and second, mitochondrial instability was detected in DCM-damaged cardiac tissue.

The Trx system forms an important junction between antioxidant defense, cell survival, and energy metabolism.[24] To the best of our knowledge, the present study is the first to describe changes of the Trx system in human HF. Several nonhuman studies underscored the central role of Trx1 in HF with the use of animal models overexpressing Trx1.[25–27] For example, studies in 2 different rodent models demonstrated that ventricular remodeling can be reduced under Trx1 overexpression.[25,26] The group of Wilson et al reported that Trx1 attenuates cardiac dysfunction in a rodent animal model of septic cardiomyopathy.[27] Previous human studies reported that serum concentrations of Trx1 increase in HF patients and correlate with disease severity.[28,29] These data are in accordance with our results. Furthermore, data from animal and human studies could lead to the hypothesis that the involvement and the role of the Trx system in HF development could be similar between mice and human. Our results showed that Trx1 expression in end-stage HF myocardium was lower in ICM but not in DCM. DCM has been shown to be affected in another part of the Trx system: the mitochondrial-specific Trx system.[30] Whereas Trx1 is part of the cytosolic Trx system, Trx2 belongs to the mitochondrial Trx system. Huang et al investigated the role of Trx2 in a spontaneous rodent DCM model by means of Trx2-knockout and found that mitochondrial Trx2 preserves cardiac function.[30]

One reason for lower Trx1 protein levels could be an impaired UPS. It is known that ischemic injury leads to UPS dysfunction and altered expression levels of E3 ligases.[12] E3 ligases are responsible for ubiquitin coupling to target proteins, and E3 ligase expression is significantly reduced at early and late stages after myocardial infarction in mice.[31] According to our results, protein expression levels for the E3 ligase ITCH were lower, causing up-regulation of TXNIP expression. Those results accord with studies reporting that ITCH was down-regulated by self-ubiquitylation[32–34] and by ubiquitin proteasomal degradation in ROS-induced cardiotoxicity.[17] Furthermore, a rodent study investigating the effects of ITCH overexpression on the Trx system reported ITCH-dependent TXNIP degradation.[17] Our results support the hypothesis that ITCH counteracts TXNIP expression under pathologic conditions in the human myocardium.

TXNIP regulates glucose homeostasis through induction of endocytosis of glucose transporter 1 and regulates mitochondrial function during ischemia via inhibition of Trx1.[24] Trx1 itself up-regulates and protects mitochondrial proteins from oxidation and dysfunction[35,36] and preserves mitochondrial function through inhibition of mitochondrial permeability transition pore (mPTP) opening.[37] TXNRD1 reduces Trx1, thereby regulating the redox state of this molecule.[38] In the present study, Trx1 levels were significantly down-regulated. In addition, TXNRD1 amount and activity were reduced, suggesting that the regeneration of Trx1 was profoundly attenuated.

The impaired Trx system leads to mitochondrial instability which was documented by greater amounts of cytochrome C in the cytoplasm of the ICM and DCM groups. Furthermore, the intrinsic apoptosis pathway was stimulated in DCM, documented by an increase of caspase-9. Caspase-3 levels were similar to those of the control group,

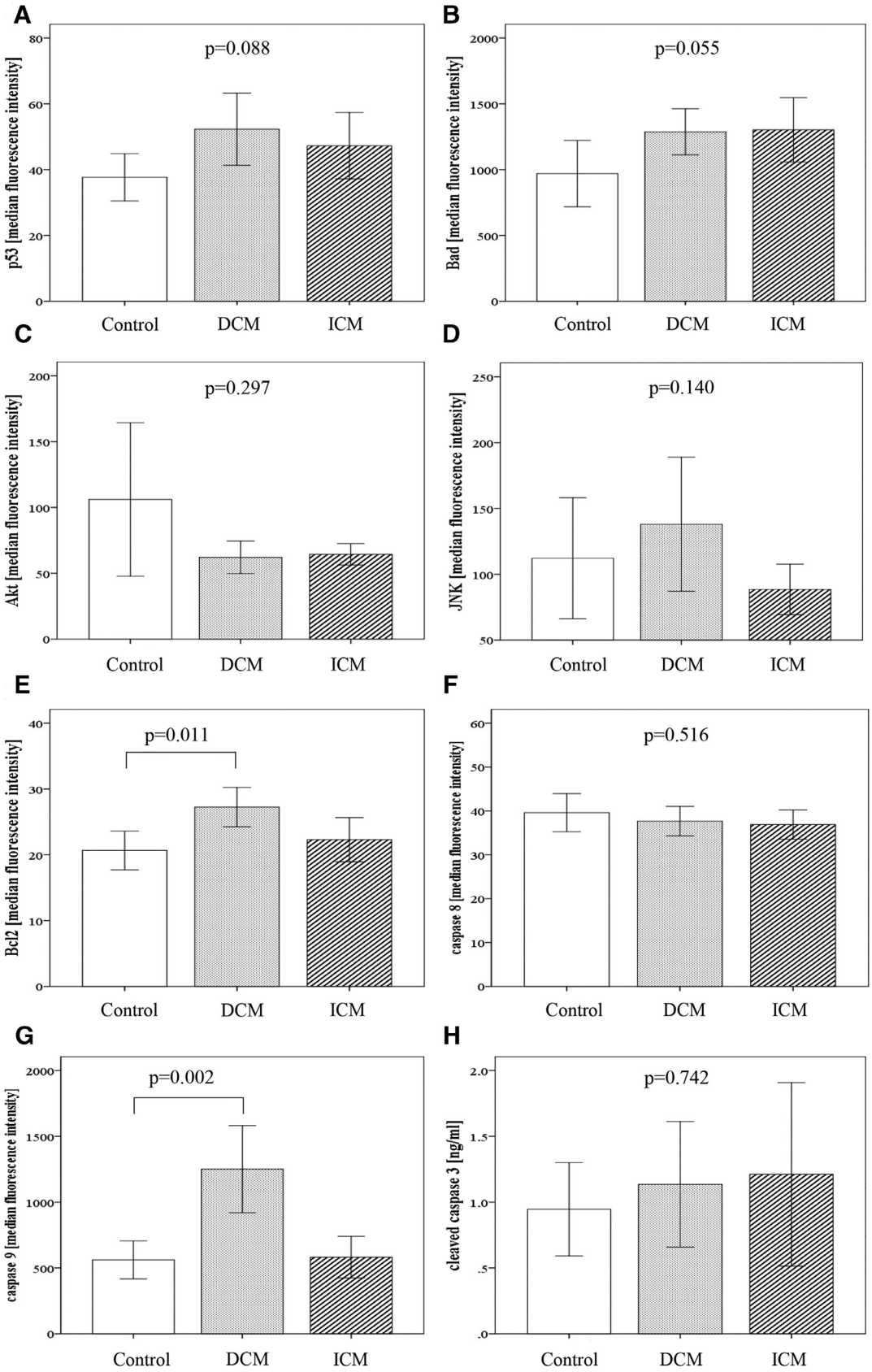

Fig. 4. Relative expression of (A) p53, (B) Bad, (C) Akt, (D) JNK, (E) Bcl-2, (F) caspase-8, (G) active caspase-9, and (H) expression of cleaved caspase-3 in myocardial tissue from control (n = 12), DCM (n = 13), and ICM (n = 13) patients as measured with the use of multiplex analysis. Akt, protein kinase B; Bad, Bcl-2−associated death promoter; Bcl2, B-cell lymphoma 2; DCM, dilated cardiomyopathy; ICM, ischemic cardiomyopathy; JNK, c-Jun N-terminal kinase; p53, tumor protein p53.

suggesting that the rate of caspase-dependent apoptosis in end-stage HF patients suffering from ICM and DCM was similar to that of control patients. The lack of apoptotic markers within the ICM group could be interpreted as follows: first, as a result of the fact that caspase-dependent apoptosis took place earlier after myocardial infarction and did not play an important role in end-stage HF in the ICM group; and second, considering that enhanced ROS production during HF is associated with less production of adenosine triphosphate (ATP), leading to ATP depletion in the cell.[39] This could happen in ischemic conditions, such as with accumulated ROS-damaged mitochondria attenuating ATP production.[40,41] Furthermore, ATP depletion is a factor that has impact on whether the apoptotic pathway or necrotic pathway is activated, favoring the necrotic pathway.[42] Therefore, it should be considered that necrosis, not apoptosis, could have taken place in the ICM group.[43,44]

As mentioned above, there was no evidence for an impaired Trx system or down-regulation of ITCH in the DCM group. It may be argued that the expected results were drawn from an animal model that was not entirely fit to simulate DCM in the human myocardium. It should be considered that the etiology of the DCM is quite complex, ranging from multigenetic to acquired causes and mixed versions thereof.[45,46] This suggests that the DCM group could be more heterogeneous than the ICM group and therefore that the mechanisms impairing the Trx system in the ICM group do not necessarily take effect in every type of DCM.

Nevertheless, mitochondrial dysfunction marked by increased cytosolic cytochrome C and significantly increased caspase-9 levels could be detected in the DCM group. The increase in Bcl-2 could be explained as a compensatory increase countering the release of cytochrome C from the mitochondria into the cytosol and the initiation of the intrinsic apoptosis pathway. However, mitochondrial dysfunction is regarded to be a general factor in DCM, and our results appeared to reflect this hypothesis. Furthermore, the hypothesis that mitochondrial dysfunction has an essential role in DCM development was supported by previous results obtained from a nonhuman study, which showed that mitochondrial knockout of Trx2 increased mitochondrial ROS production and induced spontaneous DCM.[30]

The present study was limited by a small patient number (<20 per group), which lowers the power of the study. The results enable a more reliable sample size calculation for further studies investigating changes in the UPS or the Trx system. Another limitation is the lack of a control group consisting of apex tissue from heart-healthy patients. Healthy myocardium could be obtained from tissue banks. However, the time between confirmation of death and tissue sampling ranges from several hours to days. This time frame until tissue sampling is too long and would affect the components of the UPS and the Trx system. Therefore, the present study included a control group consisting of cardiac tissue from patients without cardiomyopathy. Furthermore, group differences in clinical characteristics existed which might have affected the results of this study. First, the proportion of male subjects was significantly higher in the DCM and ICM groups compared with the control group. This can be explained by the epidemiology of the underlying pathology: the ICD-10 classified ICM as a subclass of coronary heart disease (CHD), which occurs more often in men than in women.[47,48] The same is reported for DCM.[49] Male patients are more likely to develop severe outcomes leading to end-stage HF and resulting in a higher number of ventricular assist device implantations. In contrast, severe aortic stenosis tends to increase in female patients with increasing age. Therefore, the sex difference between the study groups is a result of different sex-related prevalences. Second, the age differences between the study groups is related to the prevalence of severe aortic stenosis, which is highest in the seventh and eighth decades, and the prevalence of ICM and DCM, which are highest in sixth and fifth decades, respectively.[48−50] In addition, it has been reported that the activity of the UPS is reduced with increasing age.[51] This must be considered when the decreased level of ITCH in the ICM group is compared with the control group. The group differences observed in this study exist despite the significant higher age of the control group. A further limitation of this study is the use of end-stage HF myocardium in the ICM and DCM group, which does not allow clarifying the pathologic mechanisms. Calculation of multiplex analysis was based on normalization to the protein amount and not to cell count. This approach bears the risk that cell death signals that are cell number dependent are misinterpreted. Furthermore, we did not differentiate various types of cell death, such as caspase-independent apoptosis, necroptosis, and autophagy, and we did not perform electron microscopy which would have given further information regarding the pathways involved in Trx system impairment and its consequences. A longitudinal study design would allow monitoring of pathologic progress of UPS and Trx system impairment and would help to identify possible intervention targets to avoid ICM- and DCM-induced damage.

Conclusion

The Trx system was impaired in ICM- but not in DCM-damaged cardiac tissue. The E3 ligase ITCH of the UPS appeared to be highly responsible for the down-regulation of the Trx system, suggesting that an impaired UPS in end-stage HF tissue negatively affects the Trx system. Further studies should evaluate E3 ligases as targets for ICM therapies. Furthermore, ROS-induced mitochondrial instability appears to play a role in DCM-damaged cardiac tissue.

Disclosures

None.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.cardfail.2019.01.017.

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