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NH_4Cl treatment prevents doxorubicin-induced myocardial dysfunction *in vivo*

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ABSTRACT

Aims: Improvements in cancer treatment have significantly extended the lifespan of patients. However, due to the adverse effects of cancer treatment, cancer survivors are at increased risk of cardiovascular complications. Doxorubicin is a widely used spectrum antitumor drug, but the life-threatening side-effect of cardiotoxicity limits its clinical application. Ammonium chloride (NH_4Cl), as a heteropolar compound with pH value regulation, can cause intracellular alkalization and metabolic acidosis thus effecting enzymatic activity and influencing the process of biological system. The underlying effect of NH_4CL in DOX-induced cardiomyocyte apoptosis and hypertrophy in mice has never been reported before.

Main methods: This study we used DOX to induce cardiac remodeling and dysfunction in mice. Myocardial histology was performed using HE staining. Myocardial cell size was measured by wheat germ agglutinin (WGA) staining. Echocardiographic evaluation of cardiac function, qPCR detection of the mRNA expression of cardiac hypertrophy and inflammation markers. Apoptosis was detected by TUNEL method. Transmission electron microscopy (TEM) was used to detect autophagy.

Key findings: We found that NH_4CL effectively improved DOX-induced cardiomyocyte apoptosis and cardiac dysfunction in mice. Our results showed that NH_4CL significantly improved DOX-induced contractile dysfunction, inflammation, apoptosis and autophagy in mice.

Significance: Our results indicate that NH_4CL is effective in improving DOX-induced cardiac dysfunction and remodeling. It may therefore be a therapeutic entry point to limit doxorubicin-mediated adverse cardiac reactions.

1. Introduction

Cancer is one of the leading causes of death worldwide, with nearly 9 million deaths in 2016 [1]. The number of cancer survivors is increasing due to early detection, awareness and improvement of therapeutic interventions [2]. However, after cancer treatment, cancer survivors develop heart failure due to the cardiotoxic effects of chemotherapeutic drugs such as Doxrubicin (DOX) [3].

As an effective anthracycline antibiotic for the treatment of various cancers, DOX has been limited by cardiotoxicity, manifested as arrhythmia, arterial hypertension, thromboembolism, angina pectoris, myocardial infarction and heart failure [4,5]. The mechanism of DOX-induced cardiotoxicity is complex and has been shown to involve various signaling pathways, including free radical generation, calcium overload, mitochondrial dysfunction, apoptosis and autophagy [6,7]. However, the effective cardioprotective adjuvants to protect against DOX-induced myocardial toxicity have not been found.

Ammonium chloride (NH_4Cl) is able to induce acidosis by changing the pH value [8]. Modulated pH could effectively influence specific anti-proliferative, proapoptotic, immunomodulatory and cell-based therapies [9,10]. Evidence supports that NH_4Cl could act as a novel protective agent against kidney tissue calcification, pulmonary vascular reactivity and hypertrophic remodeling [11]. Whereas the function of NH_4CL in DOX-induced myocardial toxicity has never been studied.

Therefore, this study used a new low-dose DOX treatment model to investigate the effect of the addition of NH_4Cl on DOX cardiotoxicity in

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drinking water that causes metabolic acidosis, which can lead to moderate but progressive cardiomyopathy.

We have experimental and observational evidence that NH_4Cl can be used as a protective agent for DOX-induced myocardial cardiac hypertrophy, inflammation, oxidative stress, apoptosis and autophagy.

2. Materials and methods

2.1. In vivo model of chronic DOX-induced cardiomyopathy

We established a model that provokes modest and progressive cardiotoxicity that is reminiscent of the effects seen in patients. Chronic heart failure was induced with 5 mg/kg DOX once a week for 2 weeks in 8–9-week-old C57B/L6 mice with or without additional treatment with NH_4Cl (0.28 M in drinking water). All procedures were approved by the Institutional Animal Care and Use Committee of Dalian Medical University. The investigation was in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 2011). C57B/L6 mice were maintained on a 12-h light/dark cycle from 6 am to 6 pm and had free access to food (Sniff) and tap drinking water. Ventricular size and function were examined in acclimatized mice following the intraperitoneal administration of saturated 3-bromoethanol anaesthesia by echocardiography using a Vevo 770 ultrasound system (Visual Sonics Inc.) equipped with a 30-MHz transducer 7 days after each injection. We sacrificed the mice and performed additional experiments 7 days after the final injection. Hearts were removed from mice anaesthetized with saturated 3-bromoethanol intraperitoneally.

2.2. Quantitative PCR (qPCR)

Total RNA was isolated from heart tissues using TRIzol Reagent (Sigma Aldrich) according to the manufacturer's instructions. The levels of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), interleukin 1β (IL-1β) and Collogen I mRNA expression were measured by qPCR using an iCyclerIQ system (Bio-Rad, USA). RNA purity was determined by the absorbance value of 260/280 (1.8–2.0). Reverse transcription of 2 μg of RNA was performed using oligo (dT) primers and reverse transcriptase (Takara). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an endogenous control. The following primers were used:

Gene	Forward primer	Reverse primer
IL-1β	5′-CTTCCCCAGGGCATGTTAAG-3′	5′-ACCCTGAGCGACCTGTCTTG-3′
ANP	5′-GAGGAGAAGATGCCGGTAGA-3′	5′-AGCAGCTGGATCTTCGTAGG-3′
BNP	5′-GAAGGTGCTGTCCCAGATGA-3′	5′-CCAGCAGCTGCATCTTGAAT-3′
GAPDH	5′-GGTTGTCTCCTGCGACTTCA-3′	5′-GGTGGTCCAGGGTTTCTTACTC-3′

2.3. Histological and immunohistochemical analysis

Cardiac histology analysis was performed according to standard protocols. Heart sections were stained with hematoxylin-eosin (H&E) and wheat germ agglutinin (WGA)-TRITC conjugate. The number of macrophages in the heart sections was measured using a Mac-2 antibody or an isotype control.

2.4. TUNEL assay

Cardiomyocyte apoptosis in heart sections was assessed using a TDT-mediated dUTP nick end labeling TUNEL system (Roche) according to the manufacturer's instructions. Cardiomyocytes were identified by immunohistochemical staining with α-actinin, and the nuclei were counterstained with DAPI. Percentage of TUNEL-positive cardiomyocytes counted 10 fields per section under the microscope, and the percentage of TUNEL-positive cardiomyocytes was determined.

2.5. Caspase 3 activity

Cardiomyocyte caspase 3 activity was measured using a Caspase 3 activity assay kit (Beyotime, China) and evaluated by manual guidance. Using BD FACSCanto II as the light source, the fluorescence of cresyl violet fl was excited by a 405 nm laser. Protein concentration was determined by the Bradford method.

2.6. Transmission electron microscopy

Animals were perfused with heparinized water. The cardiac specimen was taken to be approximately 1 mm^3, fixed with 2.5% glutaraldehyde (Sigma, G7526) in 0.1 M phosphate buffer, fixed in the same buffer with 2% osmium tetroxide, dehydrated with gradient ethanol, and embedded in agar. 100 epoxy resin (Agar Science, AGR1045). Ultrathin sections were 70 nm thick, stained with 2% uranyl acetate and lead citrate, and subjected to transmission electron microscopy at 80 kV (Morgagni, FEI268D TEM, Hillsboro, USA). The image was acquired with an 11 megapixel CCD camera (Olympus-SIS, Shinjuku, Tokyo, Japan).

2.7. Statistics

We performed three parallel experiments. All data are presented as the means ± SEM. Differences between treated mice were evaluated using Student's *t*-test, and two-way ANOVA followed by the Tukey *post hoc* test was performed to compare groups with different treatments. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. NH_4Cl inhibits DOX-induced cardiomyocyte hypertrophy in mice

We know that cardiac hypertrophy is one of the important signs of cardiac remodeling. We studied the effect of NH_4Cl on cardiomyocyte hypertrophy. Heart size, cardiac weight/tibia length ratio (HW/TL), and heart weight/body weight (HW/BW) were significantly increased after 2 weeks of DOX administration in WT mice. The above changes in NH_4Cl-treated mice were inhibited after DOX treatment (Fig. 1A–B). The expression of atrial natriuretic peptide (ANF) and brain natriuretic peptide (BNP) mRNA and the increase of DOX-induced increase in cross-sectional area of myocardial cells were significantly inhibited in NH_4Cl-treated mice (Fig. 1C–D).

3.2. NH_4Cl attenuates DOX-induced cardiac inflammation

Cardiac inflammation is an important factor in the development of cardiac dysfunction under pathological conditions. Therefore, we further determined whether NH_4Cl can inhibit DOX-induced myocardial inflammation. The results showed that the infiltration of Mac-2 and $Ly6G^+$positive macrophages in the DOX group was significantly increased, and the infiltration was significantly attenuated after NH_4Cl treatment (Fig. 2A and C). After treatment with NH_4Cl, the mRNA expression level of IL-1β was significantly decreased ($P < 0.05$), but there was no significant difference compared with the control group ($P > 0.05$), and there was significant difference compared with the DOX + NH_4Cl group ($P < 0.05$) (Fig. 2B).

3.3. NH_4Cl inhibits DOX-induced cardiac injury by apoptosis in mice

To verify whether NH_4Cl inhibits DOX-induced cardiac damage and apoptosis *in vivo*, we examined the heart sections using the TUNEL method. TUNEL-positive cardiomyocytes were hardly detected in the heart of WT mice given saline. However, DOX injection significantly

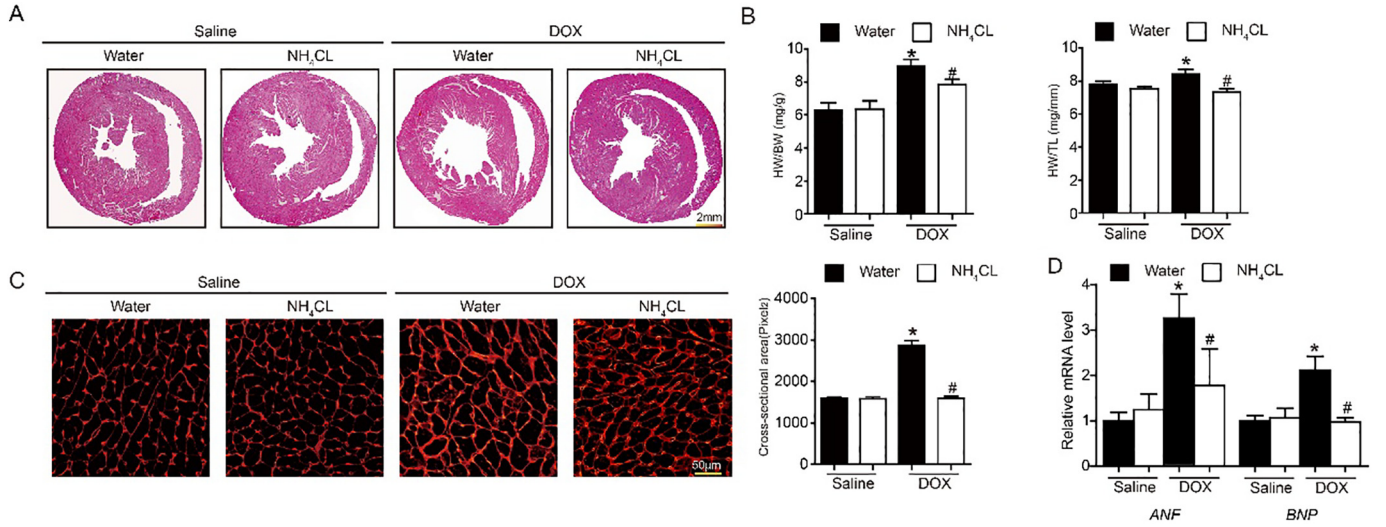

Fig. 1. NH_4Cl inhibits DOX-induced myocardial hypertrophy in mice.
(A) Histological analysis of heart sections from vehicle or NH_4Cl treated mice following control or DOX. Heart cross-sections were stained with hematoxylin-eosin Scale bar 500 μm. (B) The heart weight to tibia length ratios (HW/TL) and body weight ratios (HW/BW) in vehicle or NH_4Cl treated mice following control or DOX; (C) Heart cross-sections were stained with wheat germ agglutinin (WGA) Scale bar 50 μm; (D) Atrial natriuretic factor (ANF) and Brain natriuretic peptide (BNP) mRNA levels in the hearts of vehicle or NH_4Cl treated mice following control or DOX. * $P < 0.05$ *vs.* water group; # $P < 0.05$, *vs.* DOX plus NH_4Cl group.

increased the number of TUNEL-positive cardiomyocytes. In contrast, the number of DOX-induced TUNEL-positive cardiomyocytes was significantly reduced after treatment with NH4Cl (Fig. 3A). The activity of caspase 3 is then detected. We found that caspase3 activity was elevated in the DOX treatment group and caspase3 activity was significantly reduced after NH4Cl treatment (Fig. 3B).

3.4. NH_4Cl inhibits DOX-induced cardiac injury by autophagy in mice

Seven days after the final injection of DOX, cardiomyocyte autophagy was assessed by the observation of autophagosomes using TEM. We found that the number of autophagosomes were increased in DOX-treated group, and significantly reduced after treatment with NH_4Cl (Fig. 4).

3.5. Effect of NH_4Clon signaling pathways of cardiac hypertrophy, apoptosis and autophagy markers

Next, we further clarify whether NH_4Cl can protect the molecular mechanism of DOX-induced cardiac dysfunction and remodeling. This article mainly evaluates the expression changes of hypertrophy, apoptosis and autophagy markers. The results showed that the expression of p-AKT, AKT, p-ERK1/2, ERK1/2, PARP and Beclin 1 in the mouse heart of the DOX group was significantly increased, but the expression levels of these proteins were significantly inhibited after treatment with NH_4Cl (Fig. 5).

3.6. NH_4Cl ameliorates DOX-induced cardiac dysfunction

Seven days after the final injection of DOX, cardiac function was

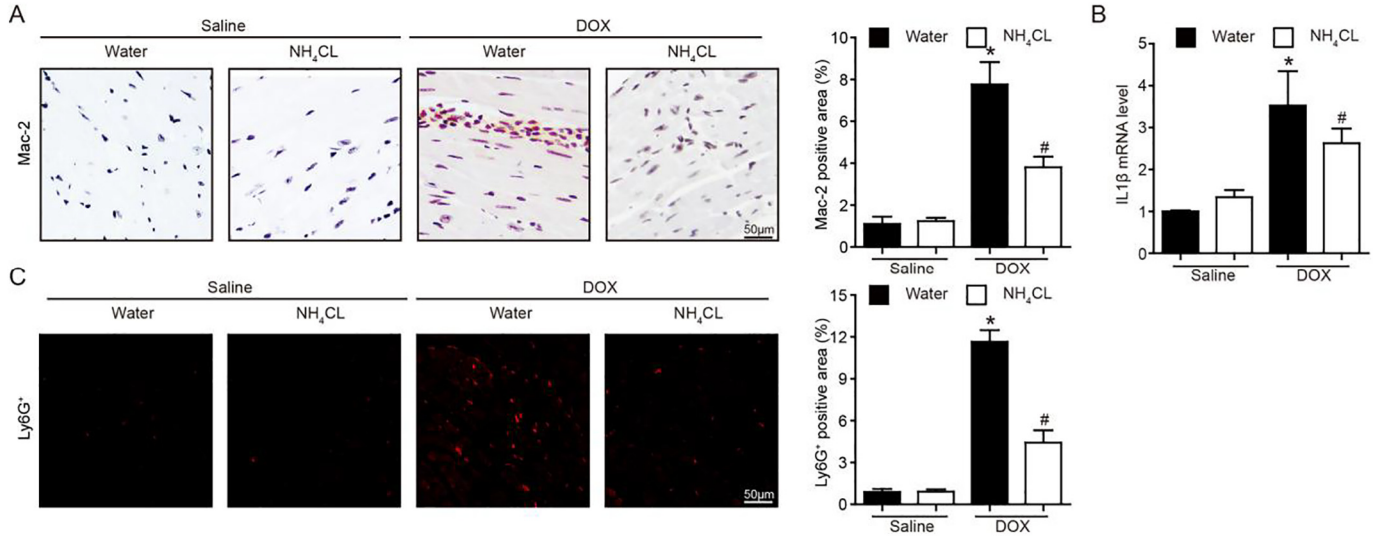

Fig. 2. NH_4Cl attenuates DOX-induced cardiac inflammation.
(A) Representative immunohistochemical staining of ventricular sections with anti-Mac-2 antibody. Scale bars: 50 μm. Quantification of Mac-2-positive cell areas (right); (B) qPCR analyses of the mRNA expressions of IL-1β. The data are normalized to the GAPDH; (C) Representative Immunofluorescence staining of ventricular sections with anti-$Ly6G^+$ antibody. Scale bars: 50 μm. Quantification of $Ly6G^+$-positive cell areas (right). The data are normalized to the GAPDH. * $P < 0.05$ *vs.* water group; # $P < 0.05$, *vs.* DOX plus NH_4Cl group.

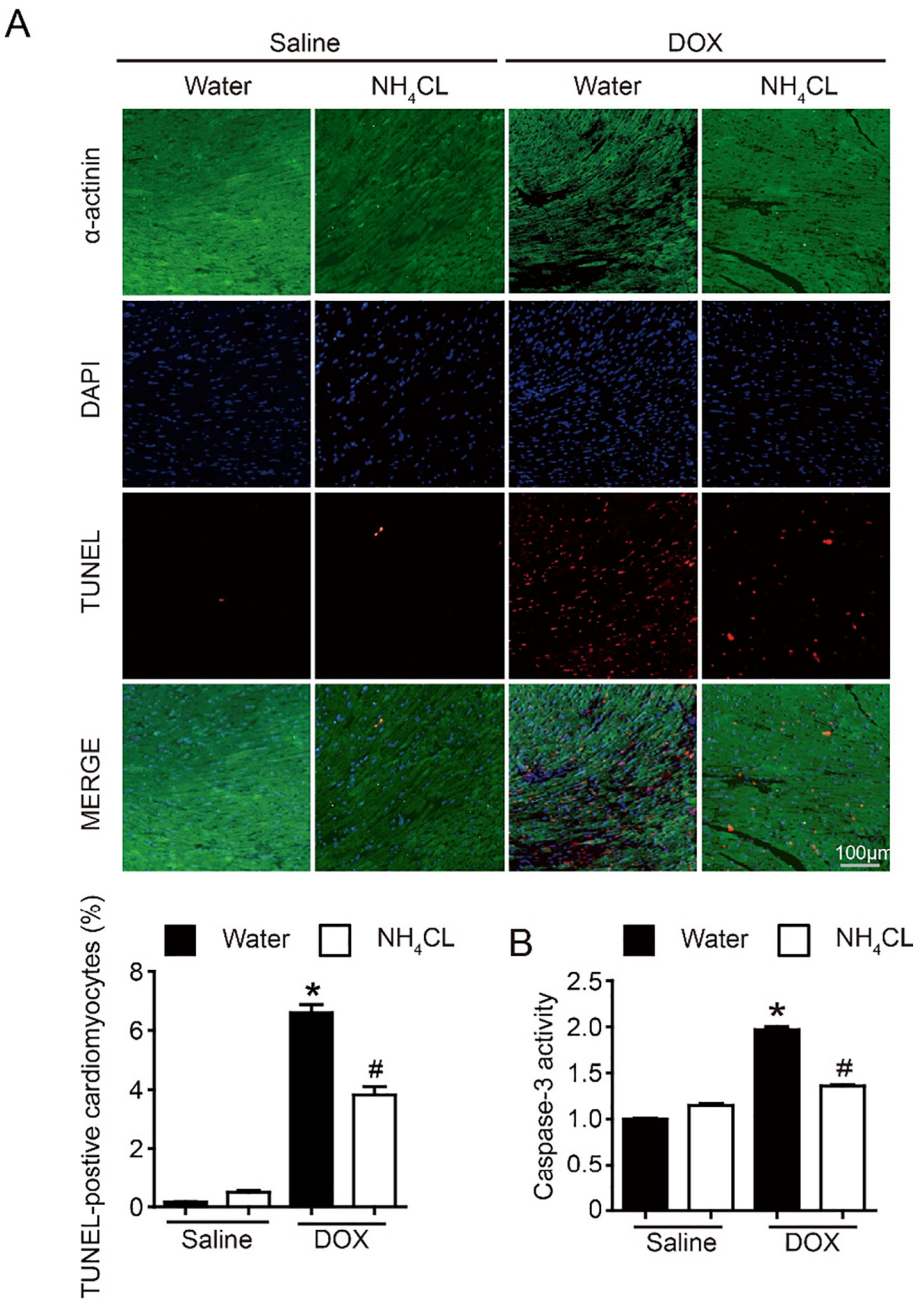

Fig. 3. NH_4Cl inhibits DOX-induced cardiac injury by apoptosis in mice.
(A) Representative TUNEL staining of cardiomyocyte apoptosis in heart sections. Quantification of TUNEL-positive nuclei (n = 10). Scale bar: 100 μm ; (B) the caspase 3 activity.

evaluated by echocardiography. We found that mice treated with NH_4Cl significantly ameliorated left ventricular (LV) contractile function as reflected by left ventricular posterior wall thickness at end-systole (LVPWs), left ventricular posterior wall thickness at end-diastole (LVPWd), left ventricular anterior wall thickness at end-systole (LVAWs), left ventricular anterior wall thickness at end-diastole (LVAWd), LV ejection fraction (EF%) and fractional shortening (FS%) compared with vehicle group after DOX treated (Fig. 6A–G).

4. Discussion

Here our study identified NH_4Cl to protect cardiomyocytes from DOX-induced heart damage *in vivo*. These data suggest that there was a functional link among NH_4Cl, DOX and heart damage. Our findings are summarized in Fig. 7.

Delayed DOX-induced cardiomyopathy has been confirmed 4 to 20 years after completion of DOX treatment. The most serious side effect of doxorubicin treatment is cardiomyopathy, followed by congestive heart failure [12]. DOX-induced heart failure is determined by the observation of left ventricular EF decreases in serial echocardiograms [13] and DOX-induced cardiomyopathy involves the rapid expansion of endothelial cell and cardiomyocyte size, which can result in apoptosis and necrosis [14].

In the present study, we observed that after the injection of DOX, the function of heart in mice had been impaired mainly representing in enlarged heart size, decreased EF and increased mRNA level of ANP as well as BNP which treated as the best prognostic indicator in all stages of heart failure compared with NS group [15]. We also firstly observed

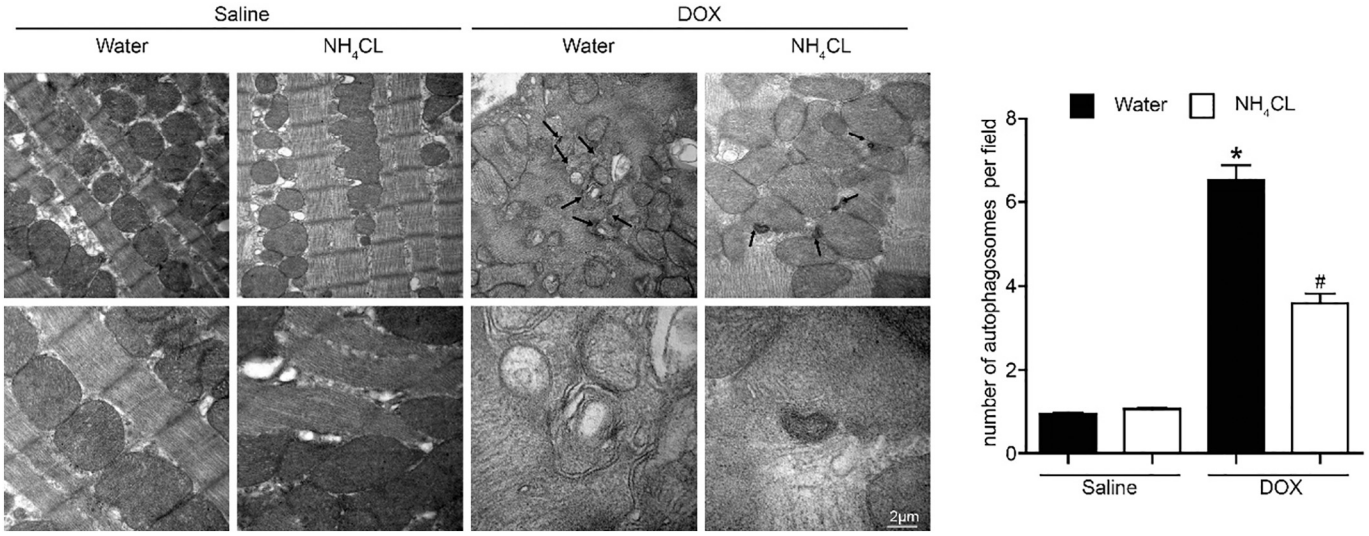

Fig. 4. NH_4Cl inhibits DOX-induced cardiac injury by autophagy in mice.
Representative images of transmission electron microscopy of myocardium ultrastructure.

that the supplementation of NH_4CL improved left ventricle dysfunction and also significantly protect the myocardium against DOX *in vivo*.

Numerous experiments have proved that DOX can trigger inflammation and cause cardiotoxicity. In this study, we found an increase in the level of interleukin-1β (IL-1β) which was agreed with previous studies [16] and the accumulation of Mac-2 and $Ly6G^+$ in cardiac myocytes after treating with DOX. The pre-treatment of NH_4CL which was able to cause metabolic acidosis in mice may limit inflammation from preventing the producing of IL-1β [17]. Furthermore, the inflammation caused by DOX could also attenuate by NH_4CL according to the previous research [18].

Of numbers of mechanism which have been presented in the toxic effect of DOX in heart, autophagy is the most controversy one. In recent years, DOX-induced cardiomyocyte autophagy has been shown to play an important role because inhibition of autophagy by chemical or genetic methods can significantly increase DOX-induced cardiomyocyte death [19,20]. Therefore, a potential therapeutic strategy to reduce DOX cardiotoxicity is to inhibit DOX-induced apoptosis and autophagy.

NH_4CL, as an autophagy inhibitor as well as ionic compound, can alkalinize acidic cellular compartments and induce metabolic acidosis. NH_4CL can transiently alkalinize acidic compartments and generate intracellular NH_3 which could rapidly diffuse across the cytoplasmic membrane and bind H^+ thus trapped as NH_4^+ in swells cells and acidic cellular compartments [21–23]. NH_4^+ may further break down into H^+ and NH_3, which can easily go across membranes, thus entering other cellular compartments and cells [24].

Consistent with the above described characteristics of cardiotoxicity, we found in the present study that the mice undergoing the DOX for 2 weeks demonstrated the increased ratios of HW/BW and HW/TL and the up-regulated mRNA expression of ANF and BNP, the specific marker of hypertrophy compared with the control. In order to study the attenuation mechanism of NH_4CL on cardiotoxicity, we evaluated cardiac hemodynamics. The results showed that NH_4CL improved EF% and FS% compared with TAC alone, suggesting that improvement in cardiac hemodynamics may explain, at least in part, the attenuation of cardiotoxicity by NH_4CL. We also found that NH_4CL has the ability to inhibit inflammation, autophagy and apoptosis.

5. Conclusion

To date, no single chemical or synthetic drug has been available to prevent the harmful effects of DOX. Therefore, finding an effective and

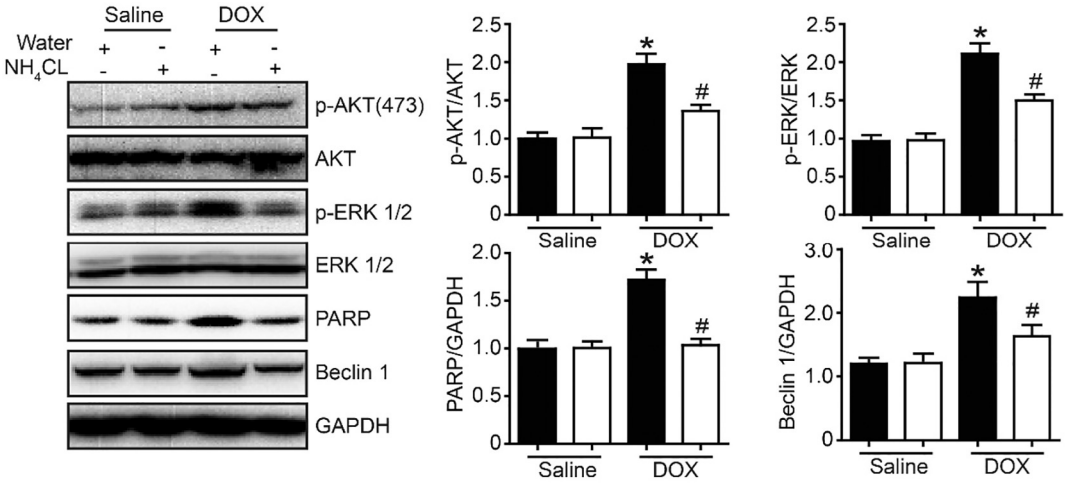

Fig. 5. Effect of NH_4Cl on signaling pathways of cardiac hypertrophy, apoptosis and autophagy markers.
Representative Western Blot analyses of p-AKT, AKT, p-ERK1/2, ERK1/2, PARP and Beclin 1 in the heart tissue. Quantification of the relative protein levels. Take GAPDH as an endogenous control.

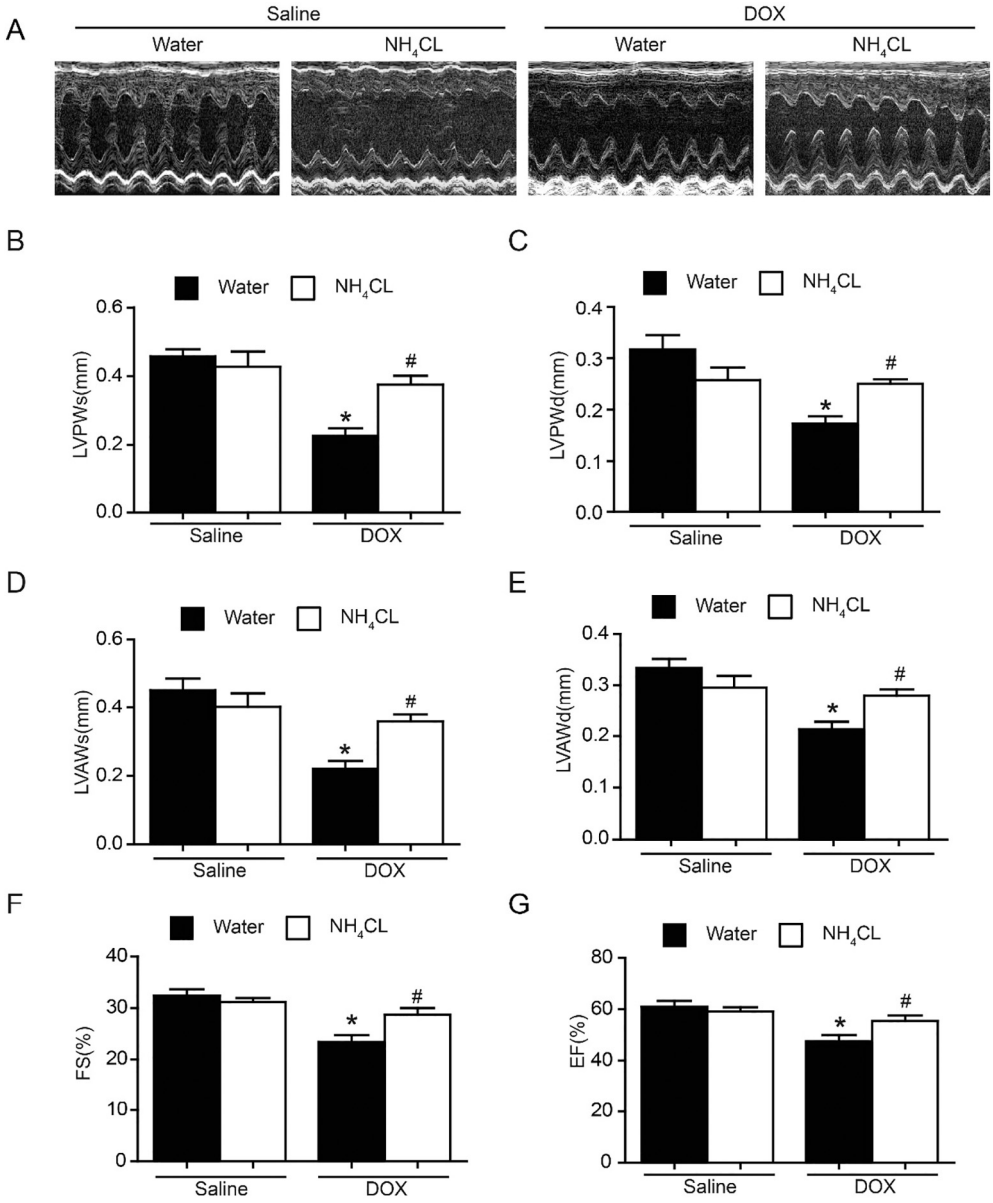

Fig. 6. NH_4Cl ameliorates DOX-induced cardiac dysfunction.
(A–G) Representative M-mode echocardiography of left ventricular chamber, and assessment of left ventricular posterior wall thickness at end-systole (LVPWs), left ventricular posterior wall thickness at end-diastole (LVPWd), left ventricular anterior wall thickness at end-systole (LVAWs), left ventricular anterior wall thickness at end-diastole (LVAWd), left ventricular ejection fraction (EF%) and fractional shortening (FS%). * $P < 0.05$ *vs.* water group; # $P < 0.05$, *vs.* DOX plus NH_4Cl group.

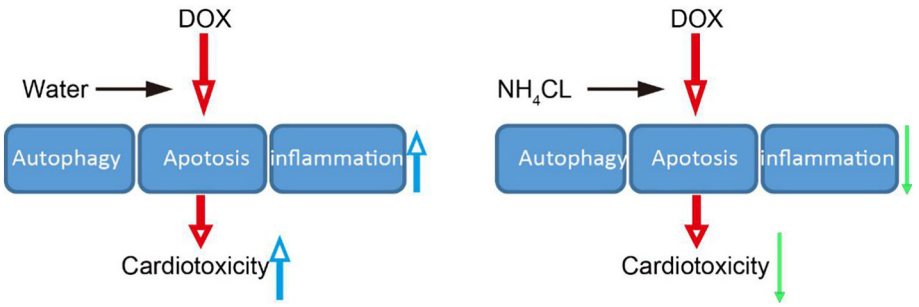

Fig. 7. A working model of NH_4Cl-mediated cardio-protection in DOX-induced cardiotoxicity.

safe DOX cardiotoxic agonist remains a challenge. In this paper, for the first time, we showed that 0.28 M NH_4Cl in drinking water is a protective agent in C57BL/6 mice during DOX injection and we concluded that NH_4Cl promotes the survival of myocardial cells *in vivo* by decreasing contractile dysfunction, cardiac hypertrophy, inflammation, apoptosis and autophagy. This observation may lead to the

development of novel therapeutic modalities.

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Disclosure statement

The authors declare that there are no conflicts of interest.

Author contributions

Xin Huang: performed experiments, analysed data. Yang Liu: performed experiments, analysed data. Xiaolei Yang: performed experiments. Song Lai: performed experiments. Jie Gu: performed experiments. Yunlong Zhang: performed experiments. Huihua Li: Conceptualization. Yunpeng Xie: Roles/Writing - original draft. Yunlong Xia: Writing - review & editing, Supervision, Funding acquisition.

References

[1] Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: a systematic analysis for the Global Burden of Disease Study 2016[J], Lancet 390 (2017) 1151–1210.
[2] K.D. Miller, R.L. Siegel, C.C. Lin, A.B. Mariotto, J.L. Kramer, J.H. Rowland, K.D. Stein, R. Alteri, A. Jemal, Cancer treatment and survivorship statistics, 2016[J], CA Cancer J. Clin. 66 (2016) 271–289.
[3] M.S. Ewer, S.M. Ewer, Cardiotoxicity of anticancer treatments[J], Nat. Rev. Cardiol. 12 (2015) 620.
[4] P. Vejpongsa, E.T. Yeh, Prevention of anthracycline-induced cardiotoxicity: challenges and opportunities[J], J. Am. Coll. Cardiol. 64 (2014) 938–945.
[5] C. Carvalho, R.X. Santos, S. Cardoso, S. Correia, P.J. Oliveira, M.S. Santos, P.I. Moreira, Doxorubicin: the good, the bad and the ugly effect[J], Curr. Med. Chem. 16 (2009) 3267–3285.
[6] E. Lorenzo, C. Ruiz-Ruiz, A.J. Quesada, G. Hernandez, A. Rodriguez, A. Lopez-Rivas, J.M. Redondo, Doxorubicin induces apoptosis and CD95 gene expression in human primary endothelial cells through a p53-dependent mechanism[J], J. Biol. Chem. 277 (2002) 10883–10892.
[7] S. Zhang, X. Liu, T. Bawa-Khalfe, L.S. Lu, Y.L. Lyu, L.F. Liu, E.T. Yeh, Identification of the molecular basis of doxorubicin-induced cardiotoxicity[J], Nat. Med. 18 (2012) 1639–1642.
[8] M. Nowik, N.B. Kampik, M. Mihailova, D. Eladari, C.A. Wagner, Induction of metabolic acidosis with ammonium chloride (NH4Cl) in mice and rats–species differences and technical considerations[J], Cell. Physiol. Biochem. 26 (2010) 1059–1072.
[9] T. Nishimura, L.T. Vaszar, J.L. Faul, G. Zhao, G.J. Berry, L. Shi, D. Qiu, G. Benson, R.G. Pearl, P.N. Kao, Simvastatin rescues rats from fatal pulmonary hypertension by inducing apoptosis of neointimal smooth muscle cells[J], Circulation 108 (2003) 1640–1645.
[10] S.R. Aber, W. Deng, R.G. Master, B.A. Bunnell, B.K. Taylor, S.N. Murthy, A.L. Hyman, P.J. Kadowitz, Intratracheal mesenchymal stem cell administration attenuates monocrotaline-induced pulmonary hypertension and endothelial dysfunction[J], Am. J. Physiol. Heart Circ. Physiol. 292 (2007) H1120–H1128.
[11] H. Christou, O.M. Reslan, V. Mam, A.F. Tanbe, S.H. Vitali, M. Touma, E. Arons, S.A. Mitsialis, S. Kourembanas, R.A. Khalil, Improved pulmonary vascular reactivity and decreased hypertrophic remodeling during nonhypercapnic acidosis in experimental pulmonary hypertension[J], Am J Physiol Lung Cell Mol Physiol 302 (2012) L875–L890.
[12] P.K. Singal, N. Iliskovic, Doxorubicin-induced cardiomyopathy[J], N. Engl. J. Med. 339 (1998) 900–905.
[13] C. Praga, G. Beretta, P.L. Vigo, G.R. Lenaz, C. Pollini, G. Bonadonna, R. Canetta, R. Castellani, E. Villa, C.G. Gallagher, H. von Melchner, M. Hayat, P. Ribaud, G. De Wasch, W. Mattsson, R. Heinz, R. Waldner, K. Kolaric, R. Buehner, B.W. Ten, N.I. Perevodchikova, L.A. Manziuk, H.J. Senn, A.C. Mayr, Adriamycin cardiotoxicity: a survey of 1273 patients[J], Cancer Treat Rep 63 (1979) 827–834.
[14] O.J. Arola, A. Saraste, K. Pulkki, M. Kallajoki, M. Parvinen, L.M. Voipio-Pulkki, Acute doxorubicin cardiotoxicity involves cardiomyocyte apoptosis[J], Cancer Res. 60 (2000) 1789–1792.
[15] A. Palazzuoli, M. Caputo, A. Calabro, R. Nuti, Clinical impact of BNP and other emerging biomarkers in heart failure evaluation and management[J], Minerva Cardioangiol. 60 (2012) 183–194.
[16] R.M. Guo, W.M. Xu, J.C. Lin, L.Q. Mo, X.X. Hua, P.X. Chen, K. Wu, D.D. Zheng, J.Q. Feng, Activation of the p38 MAPK/NF-kappaB pathway contributes to doxorubicin-induced inflammation and cytotoxicity in H9c2 cardiac cells[J], Mol. Med. Rep. 8 (2013) 603–608.
[17] M.E. Edye, D. Brough, S.M. Allan, Acid-dependent Interleukin-1 (IL-1) cleavage limits available pro-IL-1beta for caspase-1 cleavage[J], J. Biol. Chem. 290 (2015) 25374–25381.
[18] M. Feger, I. Alesutan, T. Castor, S. Mia, K. Musculus, J. Voelkl, F. Lang, Inhibitory effect of NH4Cl treatment on renal Tgfss1 signaling following unilateral ureteral obstruction[J], Cell. Physiol. Biochem. 37 (2015) 955–964.
[19] X. Wang, X.L. Wang, H.L. Chen, D. Wu, J.X. Chen, X.X. Wang, R.L. Li, J.H. He, L. Mo, X. Cen, Y.Q. Wei, W. Jiang, Ghrelin inhibits doxorubicin cardiotoxicity by inhibiting excessive autophagy through AMPK and p38-MAPK[J], Biochem. Pharmacol. 88 (2014) 334–350.
[20] T. An, Y. Huang, Q. Zhou, B.Q. Wei, S.J. Zhang R C, Yin, C.H. Zou, Y.H. Zhang, J. Zhang, Neuregulin-1 attenuates doxorubicin-induced autophagy in neonatal rat cardiomyocytes[J], J. Cardiovasc. Pharmacol. 62 (2013) 130–137.
[21] H. Ling, P. Ardjomand, S. Samvakas, A. Simm, G.L. Busch, F. Lang, K. Sebekova, A. Heidland, Mesangial cell hypertrophy induced by NH4Cl: role of depressed activities of cathepsins due to elevated lysosomal pH[J], Kidney Int. 53 (1998) 1706–1712.
[22] H. Volkl, F. Friedrich, D. Haussinger, F. Lang, Effect of cell volume on Acridine Orange fluorescence in hepatocytes[J], Biochem. J. 295 (Pt 1) (1993) 11–14.
[23] H. Pasantes-Morales, E. Vazquez-Juarez, Transporters and channels in cytotoxic astrocyte swelling[J], Neurochem. Res. 37 (2012) 2379–2387.
[24] A. Roos, W.F. Boron, Intracellular pH[J], Physiol. Rev. 61 (1981) 296–434.