NH4Cl Treatment Prevents doxorubicin induced myocardial dysfunction in mice meanwhile enhances doxorubicin-induced apoptosis in HeLa cells

Xin Huang 1, Xiao-Lei Yang 1, Hui-Hua Li1, Yun-Long Xia1*

1Department of Cardiology, First affiliated Hospital of Dalian Medical University, Dalian 116011, China

*Correspondence to Yun-Long Xia, MD, PhD, Department of Cardiology, First affiliated Hospital of Dalian Medical University, Dalian 116011, China.
Tel: +86-411-83632383. E-mail: yunlong_xia@126.com

Key Words Chronic heart failure • NH4Cl • HeLa • Apoptosis

ABSTRACT

Background/Aims: Chronic heart failure is the most prevalent complications induced by doxorubicin (DOX). Histopathological changes include interstitial myocardial fibrosis and vacuolated cardiomyocytes, inflammatory aggregation. According to recent observations, autophagy and apoptosis play important roles in doxorubicin induced cardiotoxicity. Ammonium chloride (NH4Cl) as a well-known inhibitor of the late stage of autophagy can also prevented phosphate-induced vascular remodeling involving decrease of Tgfβ1 expression as well as inhibition of Tgfβ1-dependent signaling. The present study, thus, explored whether NH4Cl influences doxorubicin-induced chronic heart failure meanwhile enhances doxorubicin-induced apoptosis in HeLa cells in vitro.

Methods: Doxorubicin-induced cardiotoxicity model was established by intraperitoneal administration of doxorubicin (2 injections within 2 weeks: total dose = 10 mg/kg). The C57Bl6 mice were randomly divided into dox-null group, dox group, NH4CL group and dox+NH4Cl group (0.28 M NH4Cl in drinking water, n = 6 each). After 2 weeks, cardiac function was determined by echocardiography. Histopathological changes were determined by HE, Masson, Immunohistochemistry, WGA. Transcript levels were determined by RT-PCR as well as protein abundance by Western blotting. Apoptosis rate was evaluated by caspase-3 activities, TUNEL and expression of Bcl-2, Bax. The similar parameters were obtained from Hela cells treated with NH4CL and/or doxorubicin.

Results: Two weeks after administration, LVEF and LVFS were significantly decreased in doxorubicin group as compared with control group [LVEF: (47.18±6.97)% vs. (61.91±4.57)% , P < 0.05; LVFS: (23.42±4.20)% vs. (32.45±3.01)%, P < 0.05], while LVEF and LVFS were significantly increased in dox+NH4CL group as compared with doxorubicin group [LVEF: (55.98±7.75)% vs. (47.18±6.97)% , P < 0.05; LVFS: (28.67±5.26)% vs. (23.42±4.20)% , P < 0.05]. Western blot analysis revealed higher expression of BAX as compared with dox-null controls [(3.26±2.84)% vs 1 ± 0.67 %, P < 0.05] and TUNEL [(3.83±0.65)% vs (6.6 ±0.69)% , P < 0.05] and caspase3 enzymatic activity [(13.59 ± 2.51)% vs (19.69±0.69)% , P < 0.05] showed that NH4Cl treatment significantly inhibited the apoptosis of
cardiomyocytes comparing with doxorubicin group. H&E [(4.31 ± 0.79)% vs (11.19 ± 0.81)% , P < 0.05], MASSON staining [(5.32 ± 0.38)% vs (10.8±0.67)% , P < 0.05] as well as WGA [(1589.7 ± 119.1) vs (2871.9±343.1) pixel² , P < 0.05] showed that NH4CL may attenuate the histopathological changes induced by doxorubicin. The mRNA levels of the transcription factors BNP and ANP were up-regulated in the heart tissues following injection of doxorubicin, effects again significantly ameliorated following NH4Cl treatment. Furthermore, NH4Cl increased Bax protein abundance [(1.96 ± 0.75)% vs (2.42 ± 0.84)% , P < 0.05] in HeLa cells. 

**Conclusion:** NH4Cl treatment ameliorates doxorubicin induced myocardial dysfunction in mice meanwhile enhances doxorubicin-induced apoptosis in HeLa cells in vitro.