GW23-e1034 **ROLE OF HMGB1 IN DOXORUBICIN-INDUCED MYOCARDIAL APOPTOSIS AND ITS REGULATION PATHWAY**

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Objectives Doxorubicin (DOX) is a widely used anti-tumour agent. The clinical application of the medication is limited by its side effect which can elicit myocardial apoptosis and cardiac dysfunction. However, the underlying mechanism by which DOX causes cardiomyocyte apoptosis is not clear. The aim of present study is to investigate the role of high-mobility group box 1 (HMGB1) in DOX-induced myocardial injury, and signal pathway involved in regulation of HMGB1 expression in cardiomyocytes with DOX.

Methods Cardiac myocytes were treated with DOX (0.5 μM). At the time indicated, cardiomyocytes and cell culture medium were collected. Expression of HMGB1 and released HMGB1 by cardiomyocytes was analysed by western blot and ELISA, respectively. Cardiac myocytes were challenged with DOX with or without A-box (10 μg/ml) for 6 or 24 h, myocyte apoptosis was determined by caspase 3 and cell death ELISA. Cardiac myocytes derived from wild type and TLR4 deficient mice were challenged with DOX for 6 or 24 h, and the control myocytes were treated with vehicle. Cardiac myocytes were treated with DOX. At the time indicated, the $ONOO^-$ was detected with DHR123 (10 μM) and activation of JNK was detected by phosphorylation of JNK. ONOO- was inhibited with FeTPPS and phosphorylation of JNK was inhibited by SP600125 and JNK gene mice. The expression of HMGB1 were analysis by Western blot and IHC.

Results We found treatment of isolated cardiomyocytes and naive mice with the DOX resulted in an increased HMGB1 expression which was associated with increased myocardial cell apoptosis. Pharmacological (A-box) or genetic blockade (TLR4 deficiency, $TLR4^{-/-}$) of HMGB1 attenuated the DOX-induced myocardial apoptosis and cardiac dysfunction. In addition, our study showed that DOX resulted in an increment in the generation of peroxynitrite ($ONOO^-$) and an elevation in phosphorylation of c-Jun N-terminal kinase (JNK). Pretreatment of myocytes with FeTPPS, a peroxynitrite decomposition catalyst, prevented DOX-induced JNK phosphorylation, HMGB1 expression, myocardial apoptosis and cardiac dysfunction. Genetic ($JNK^{-/-}$) or pharmacological (SP600125) inhibition of JNK ameliorated the DOX-induced HMGB1 expression and diminished myocardial apoptosis and cardiac dysfunction. Taken together, our results indicate that HMGB1 mediates the myocardial injury induced by DOX and $ONOO^-$/JNK is a key regulatory pathway of myocardial HMGB1 expression induced by DOX.

Conclusions In the previous study, we have demonstrated that HMGB1 play an important role in cardiac ischaemia/reperfusion injury and LPS-induced injury. In this study, we investigated the role of HMGB1 in DOX-induced cardiomyopathy. Our results show that DOX can induce expression of HMGB1 in cardiomyocytes which lead to the apoptosis of cardiomyocytes.

The regulatory pathways involved in the HMGB1 expression differ in different conditions. We have previously reported that TLR4/PI3Kγ pathway regulates myocardial HMGB1 expression in sepsis. However, others have demonstrated that the peroxynitrite contributes to the HMGB1 up-regulation in infracted myocardium. In the present study, our results indicate that DOX-induced HMGB1 expression is regulated by ONOO- production and JNK activation. Our results suggested that inhibition of HMGB1 could provide a means of reducing DOX-induced cardiomyopathy.