Basic science
Experimental research

[gw22-e0950] **NITRATIVE INACTIVATION RESISTANT HUMAN THIOREDOXIN-1 Y49F MUTANT STRENGTHENS THE INHIBITION OF ASK-1 MEDIATED APOPTOSIS**

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10.1136/heartjnl-2011-300867.1

Background Thioredoxin-1 (Trx) is an important antioxidant and antiapoptotic small molecule and its activity can be altered by modification on different amino acid sites. After myocardial ischemia/reperfusion (MI/R), the increase of oxidative stress stimulates nitrative inactivation of Trx. In turn, nitrated Trx intensifies MI/R injury by mediating postischemic myocardial apoptosis. However, the precise mechanism of how nitrated Trx fails to exert a cardioprotective effect is to be determined.
Methods and results In this study, we examined if Y49 is the nitrative modification site and whether the single mutation of Trx at site Y49 (Trx Y49F) can resist nitrative inactivation and strengthen the inhibition of ASK-1 mediated apoptosis. In contrast to Trx, in vitro exposure of Trx Y49F to SIN-1 (a protein nitration reagent) did not result in protein nitration. The interactions of Trx/Trx Y49F and ASK-1 were studied by cotransfection of Trx/Trx Y49F and ASK-1 plasmids into HEK293A cells. By treating cells with H_2O_2 of adequate concentration, Trx-ASK-1 complex formation was reduced and activity of ASK-1 and p38 mitogen-activated protein kinase (MAPK) increased. In contrast, mutation Y49 prevented Trx nitration, increased Trx activity, restored Trx-ASK-1 interaction, reduced ASK-1 and P38 MAPK activity, and attenuated caspase 3 activation ($p<0.01$) under the same treatment of H_2O_2. Further animal experiments confirmed that compared with Trx, Trx Y49F is more effective on protecting MI/R injury by enhancing Trx-ASK-1 interaction, inhibiting p38 MAPK signalling pathway, and subsequent decreasing postischemic cardiomyocyte apoptosis ($p<0.05$).
Conclusions Our results demonstrated that the increase of oxidative stress will result in nitration of Trx at site Y49. Nitrative inactivation of Trx can be prevented by Y49F mutation, which strengthens the inhibition of ASK-1 mediated pathological apoptosis. This finding may lead to a novel therapeutic method to attenuate MI/R injury in patients.