Mechanistic Study on Cardiovascular Protective Effects of A Novel Danshensu Derivative

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Doctor of Philosophy in Biomedical Sciences

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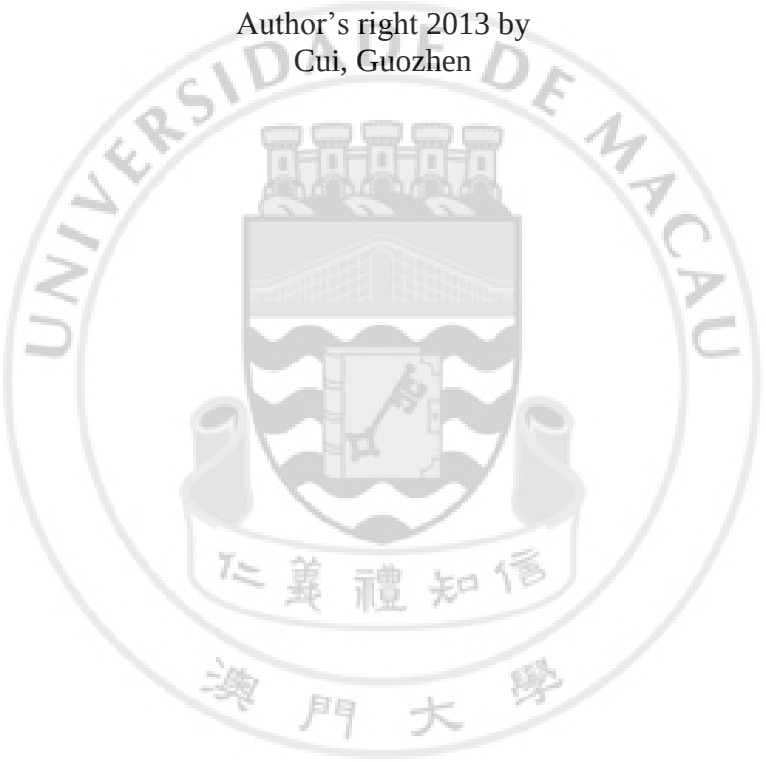

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Abstract

Cardiovascular disease (CVD) is the leading cause of global mortality and its prevalence is still increasing. Despite current drug treatments that can significantly improve the clinical outcomes in patients with CVD, adverse effects or no pharmacological responses exist in some cases. Hence, there remains an unmet need for developing novel pharmacological agents with improved efficacy and free of the side effects to prevent CVD.

Danshensu (DSS) and tetramethylpyrazine (TMP) are major active ingredients from danshen and chuanxiong, respectively. Previous studies indicated that additive synergistic effects may exist when danshen and chuanxiong are co-administered. In view of these results, we postulated that the beneficial effects of danshen and chuanxiong might be exploited by the combination of the pharmacophoric moieties of DSS and TMP. We had synthesized a novel compound named ADTM by modifying a heterodimer of DSS and TMP. The activities of ADTM were investigated in numerous experimental models of cardiovascular diseases.

ADTM was much more potent than its parent compounds, DSS and TMP alone as well as in combination, against *tert*-butylhydroperoxide-induced cell injury in H9c2 cardiomyoblasts. ADTM treatment significantly alleviated myocardial infarction in a rat model of myocardial ischemia. Moreover, the PI3K/Akt and Nrf2 pathways were found to be involved in the cardioprotective effect of ADTM both *in vitro* and *in vivo*. In addition, ADTM demonstrated a more potent and broader spectrum of anti-platelet aggregation activity than its parent compounds in *in vitro* and *in vivo*. In order to identify the protein interacting targets of ADTM, pull-down proteins bound with

biotinylated modified ADTM chemical structure were analyzed by LC-MS/MS. We had identified and confirmed protein isomerase disulfide (PDI) family members, particular ERp57, as novel interacting targets of ADTM. The mechanisms underlying the anti-platelet aggregation effect of ADTM, at least in part, are mediated by the inhibiting the activity of ERp57 as well as activating vasodilator-stimulated phosphoprotein (VASP) and heme oxygenase-1 (HO-1), leading to suppress platelets surface expression of αIIbβ3 and P-selectin.

Collectively, ADTM, a novel heterodimer of DSS and TMP, represents a promising lead candidate for the treatment of CVDs, partly through alleviating myocardial infarction and thrombotic problems.

Declaration

I declare that the thesis here submitted is original except for the source materials explicitly acknowledged and that this thesis as a whole, or any part of this thesis has not been previously submitted for the same degree or for a different degree.

I also acknowledge that I have read and understood the Rules on Handling Student Academic Dishonesty and the Regulations of the Student Discipline of the University of Macau.

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List of Abbreviations

AA	Arachidonate
ADP	Adenosine diphosphate
CVD	Cardiovascular disease
DSS	Danshensu
ER	Endoplasmic reticulum
ERp	Endoplasmic reticulum protein
FBS	Fetal bovine serum
FDA	Food and Drug Administration
HO-1	Heme oxygenase 1
IHD	Ischemic heart disease
IP	Immunoprecipitation
LDH	Lactate dehydrogenase
MS	Mass spectrometry
Nrf2	Nuclear factor erythroid 2-related factor 2
PDI	protein disulfide isomerase
PI3K	Phosphoinositide 3-Kinase
PPP	Platelet-poor plasma
PRP	Platelet-rich plasma
ROS	Reactive oxygen species
siRNA	Small interfering RNA
t-BHP	*tert*-Butylhydroperoxide
TMP	Tetramethylpyrazine
VASP	Vasodilator stimulated phosphoprotein