CaMKII inhibition as a novel target for flavonol cardioprotection

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3′,4′-dihydroxyflavonol (DiOHF) is cardioprotective against ischemia/reperfusion (I/R) injury although the molecular mechanisms underlying this beneficial effect is undetermined. The biological activities of flavonols are associated with kinase modulation to alter cell signalling events in addition to anti-oxidant and vasodilatory functions. Previously, we demonstrated that DiOHF inhibited stress-activated protein kinase (JNK, p38) signalling and enhanced the viability of cardiomyocytes challenged with oxidative stress. In order to characterize drug mechanism of action and identify the direct protein targets of DiOHF, we conducted small-molecule affinity purification and protein identification by mass spectrometry and demonstrated Ca^{2+}/calmodulin-dependent protein kinase II delta (CaMKIIδ), the cardiac predominant isoform, as a DiOHF targeted kinase. We confirmed DiOHF inhibition of CaMKII activity in various cultured cardiac cell models and *in vivo* in perfused hearts. Biochemical characterization with *in vitro* purified kinase indicated that DiOHF attenuated CaMKII activity in an ATP-competitive manner and a comparison with structurally analogous flavonols revealed the functional groups on DiOHF required for CaMKII inhibitory activity. Furthermore, we found that specific inhibition of CaMKII with KN93, but not KN92, completely attenuated oxidative stress-induced activation of JNK and p38MAPK. Taken together, our studies indicate DiOHF inhibition of CaMKII occurs upstream of stress-activated protein kinases and may be required for the protective effects of DiOHF against stress stimuli and myocardial I/R injury.