# Role of Omi/HtrA2 in Apoptotic Cell Death After Myocardial Ischemia and Reperfusion

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***Background***—Omi/HtrA2 is a proapoptotic mitochondrial serine protease involved in caspase-dependent as well as caspase-independent cell death. However, the role of Omi/HtrA2 in the apoptotic cell death that occurs in vivo under pathological conditions remains unknown. The present study was designed to investigate whether Omi/HtrA2 plays an important role in postischemic myocardial apoptosis.

***Methods and Results***—Male adult mice were subjected to 30 minutes of myocardial ischemia followed by reperfusion and treated with vehicle or ucf-101, a novel and specific Omi/HtrA2 inhibitor, 10 minutes before reperfusion. Myocardial ischemia/reperfusion significantly increased cytosolic Omi/HtrA2 content and markedly increased apoptosis. Treatment with ucf-101 exerted significant cardioprotective effects, as evidenced by less terminal dUTP nick end-labeling staining, a lower incidence of DNA ladder fragmentation, and smaller infarct size. Furthermore, treatment with ucf-101 before reperfusion attenuated X-linked inhibitor of apoptosis protein degradation and inhibited caspase-9 and caspase-3 activities.

***Conclusion***—Taken together, these results demonstrate for the first time that ischemia/reperfusion results in Omi/HtrA2 translocation from the mitochondria to the cytosol, where it promotes cardiomyocyte apoptosis via a protease activity–dependent, caspase-mediated pathway. **(*Circulation*. 2005;111:90-96.)**

**Key Words:** apoptosis ■ myocardial infarction ■ reperfusion



Growing evidence indicates that apoptosis plays an important role in the pathogenesis of a variety of cardiovascular diseases, including heart failure and ischemia followed by reperfusion.[1,2] Apoptotic cell death is mainly orchestrated by a family of aspartate-specific cysteine proteases known as caspases, the activation of which is controlled by 2 distinct pathways: the death receptor pathway (caspase-8/caspase-3 pathway) and the mitochondrial pathway (caspase-9/caspase-3 pathway).[3] Under physiological conditions, both caspase-9 and caspase-3 are thwarted by inhibitor of apoptosis proteins (IAPs), a family of cytosolic proteins that block apoptosis at a postmitochondrial level.[4] Studies in cultured cells and isolated mitochondria have demonstrated that besides cytochrome *c* and procaspases, mitochondria contain a number of other proapoptotic molecules that are released during apoptosis. Among them, the second mitochondria-derived activator of caspases (Smac)/direct inhibitor of apoptosis-binding protein with low pi (DIABLO) has been shown to play a critical regulatory role in apoptosis.[5] In mammals, Smac/DIABLO uses a conserved amino-terminal sequence (tetrapeptide motif AVPS) to bind to X-linked inhibitor of apoptosis protein (XIAP) and results in their displacement from activated caspases, thus promoting caspase-dependent apoptosis.[6]

Several recent studies[7–11] have demonstrated that Omi/HtrA2, a serine protease, promotes apoptosis by mechanisms similar to those attributed to Smac/DIABLO. Omi/HtrA2 is formed as a precursor that translocates to the mitochondria, where it is processed to its mature form by proteolytic cleavage. This processing removes an amino-terminal domain (amino acids 1 to 133) and exposes the AVPS motif. After an apoptotic insult, Omi/HtrA2 is released to the cytosol, where it interacts with XIAP and promotes caspase-dependent apoptosis. Unlike Smac/DIABLO, whose antiapoptotic property relies entirely on its physical binding with IAPs, Omi/HtrA2 stimulates apoptosis by its protease activity in addition to its physical interaction with IAPs.[12]

Although in vitro studies have demonstrated that overexpressing Omi/HtrA2 in cultured cells results in cell death

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through apoptosis, the role of normally expressed Omi/HtrA2 in vivo in apoptotic cell death has not been previously determined. In addition, removal of Omi/HtrA2 by RNA interference has been shown to reduce UV light–induced apoptosis in U2OS cells.[9] However, whether inhibition of Omi/HtrA2 attenuates apoptosis under real pathological conditions, such as in vivo myocardial ischemia/reperfusion (MI/R), has not been previously investigated. In a recent study, Cilenti and colleagues[13] demonstrated that treatment with ucf-101, a compound that shows high selectivity against the serine protease activity of Omi/HtrA2 (median inhibitory concentration for Omi/HtrA2 was 9.5 μmol/L and for other proteases, 200 to 500 μmol/L), markedly inhibited apoptotic cell death in caspase-9–null fibroblasts, suggesting that the proteolytic activity of Omi/HtrA2 plays a critical role in its proapoptotic activity. However, whether Omi/HtrA2 may promote apoptotic cell death in vivo by a similar mechanism remains unclear.

The aims of the present study were (1) to determine whether Omi/HtrA2 is translocated from the mitochondria to the cytosol in cardiomyocytes subjected to MI/R in vivo; (2) if so, to investigate whether this translocation contributes to cardiomyocyte apoptosis after I/R; and (3) to identify potential mechanisms by which Omi/HtrA2 may promote myocardial apoptosis after MI/R.

## Methods

### Materials

The investigations conformed to the *Guide for the Care and Use of Laboratory Animals* protocol, published by the US National Institutes of Health ([NIH] publication No. 85-23, revised 1985) and were approved by the Thomas Jefferson University Committee on Animal Care.

### Experimental Protocol

Adult male mice (20 to 25 g) were anesthetized with 2% isoflurane, and myocardial ischemia (30 minutes) and reperfusion (3 hours for apoptosis determination and 24 hours for myocardial infarct measurement) were performed as described in our recent study.[14] At the end of the reperfusion time the heart was quickly removed, and myocardial apoptosis (I/R area) and myocardial infarct size were determined as described later. Sham-operated animals were subjected to the same surgical procedures, except that the suture under the left anterior descending coronary artery was not tied. Ten minutes before reperfusion, animals were randomized to receive vehicle or ucf-101 (0.6 to 1.8 μmol/kg IP; estimated plasma concentrations, 10 to 30 μmol/L). ucf-101 has been shown to inhibit 50% to 90% of Omi/HtrA2 activity but has no effect on other proteases at these concentrations.[13]

### Quantification of Mitochondrial Omi/HtrA2 and Cytochrome *c* Release

Mitochondrial Omi/HtrA2 and cytochrome *c* release was determined as described by Ott et al,[15] with modifications as described in our recent study.[16]

### Determination of Myocardial Apoptotic Death

At the end of 3 hours of reperfusion, the heart was removed as described earlier. Myocardial apoptosis was qualitatively analyzed by detection of DNA fragmentation (DNA ladders) and quantitatively analyzed by terminal dUTP nick end-labeling (TUNEL) assay as described previously.[17] Assays were performed in a blinded manner.

### Assessment of AAR and Infarct Size

At the end of the 24-hour reperfusion period, the ligature around the coronary artery was reoccluded through the previous ligation. The I/R area (area at risk [AAR]) was identified by negative Evans blue dye staining, and the infarcted area was identified by negative triphenyltetrazolium chloride (TTC) staining. The Evan's blue–stained area (area not at risk), TTC-stained area (ischemic but viable tissue), and TTC-negative staining area (infarcted myocardium) were digitally measured with an IPLab 3.6. Myocardial infarct size was expressed as a percentage of infarct area divided by total AAR.

### Measurement of Caspase Activity

Myocardial caspase-3 and caspase-9 activity was determined with colorimetric assay kits (Chemicon International, Inc) according to the manufacturer's instructions. Results were expressed as μmol pNA/mg protein

### Statistical Analysis

All values in the text and figures are presented as mean±SEM of n independent experiments. All data (except Western blot density) were subjected to ANOVA followed by the Bonferroni correction as a post hoc test. Western blot densities were analyzed with the Kruskal-Wallis test followed by Dunn's post hoc test. Probabilities of 0.05 or less were considered statistically significant.

## Results

### Omi/HtrA2 Is Translocated From the Mitochondria to the Cytosol Along With Cytochrome *c* After MI/R

Recent in vitro studies have shown that cytotoxic stimulation, such as UV irradiation, results in Omi/HtrA2 translocation from the mitochondria to the cytosol. However, direct evidence demonstrating that Omi/HtrA2 is released under real pathological conditions, such as I/R in vivo, is lacking. As illustrated in Figure 1A, Omi/HtrA2 was detected in the mitochondria but not in the cytosolic fraction in tissues obtained from a sham MI/R heart. In contrast, cytosolic Omi/HtrA2 was markedly increased in myocardial tissue obtained from animals that had undergone ischemia (30 minutes) and reperfusion (3 hours). To further assess the role of Omi/HtrA2 in postischemic myocardial apoptosis, we determined the time course of Omi/HtrA2 and cytochrome *c* release after MI/R. As summarized in Figure 1B, the time course of Omi/HtrA2 release in the I/R heart closely followed cytochrome *c* release. Specifically, 30 minutes of ischemia alone resulted in neither significant Omi/HtrA2 nor cytochrome *c* release. In contrast, both Omi/HtrA2 and cytochrome *c* contents were increased after reperfusion and reached their maximal levels 120 minutes after reperfusion.

### Inhibition of Omi/HtrA2 Exerted a Significant Cardioprotective Effect Against MI/R Injury

Having demonstrated that I/R caused Omi/HtrA2 translocation, we then sought to determine whether Omi/HtrA2 after translocation plays an important role in postischemic myocardial apoptosis. For this we used ucf-101, a novel and specific inhibitor that is able to block the protease activity of Omi/HtrA2 both in vivo and in vitro. In myocardial tissue from the sham MI/R group, a very low level of TUNEL-positive staining was observed. In contrast, a significant number of TUNEL-positive cells were observed in myocardial tissue from the vehicle-treated hearts subjected to I/R.

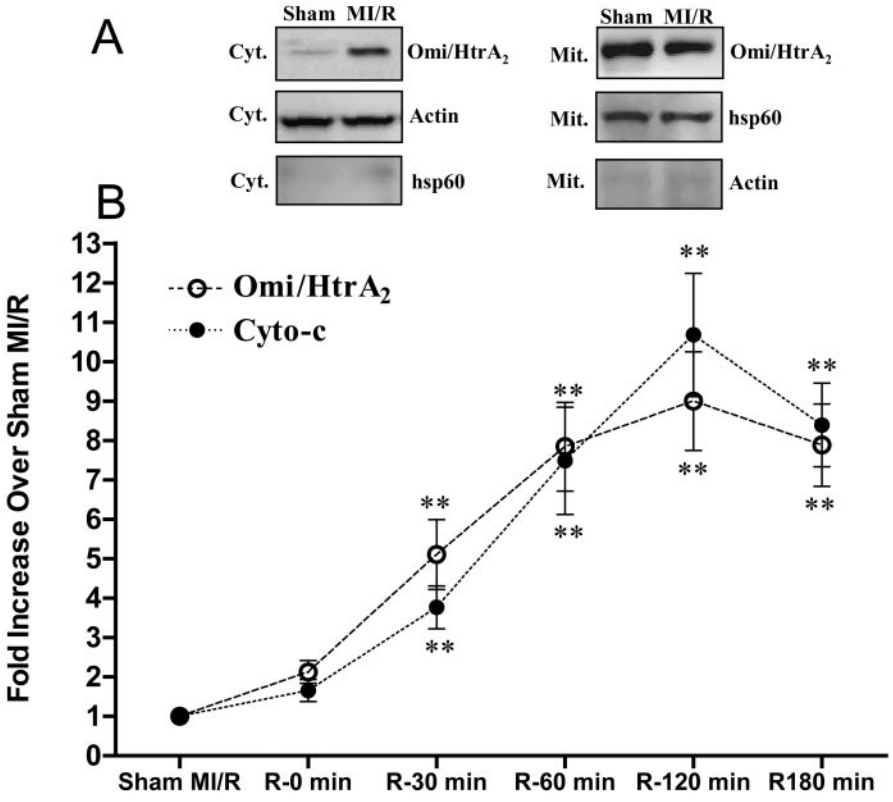


**Figure 1.** A, Representative Western blots showing that MI/R resulted in mitochondrial Omi/HtrA2 release (120-minute reperfusion). Cyt indicates cytosolic extract; Mit, mitochondrial extract; and hsp, heat shock protein. B, Time course of cytosolic (Cyto) Omi/HtrA2 and cytochrome *c* increase after ischemia followed by reperfusion. Cytosolic Omi/HtrA2 and cytochrome *c* from at least 5 animals per time point were determined by Western blot, and blot densities were determined by use of Kodak 1D analyzing software. Results are expressed as fold increase over mean density of sham MI/R. $^{**}P<0.01$ vs sham MI/R. All other abbreviations are as defined in text.

Administration of ucf-101 shortly before reperfusion reduced postischemic myocardial apoptosis in a dose-dependent fashion, with the maximal protective effect observed at a dose of 1.5 μmol/kg (Figure 2A and 2B). This dose of ucf-101 (ie, 1.5 μmol/kg) was used in all subsequent experiments. To further establish that inhibition of Omi/HtrA2 protease activity led to

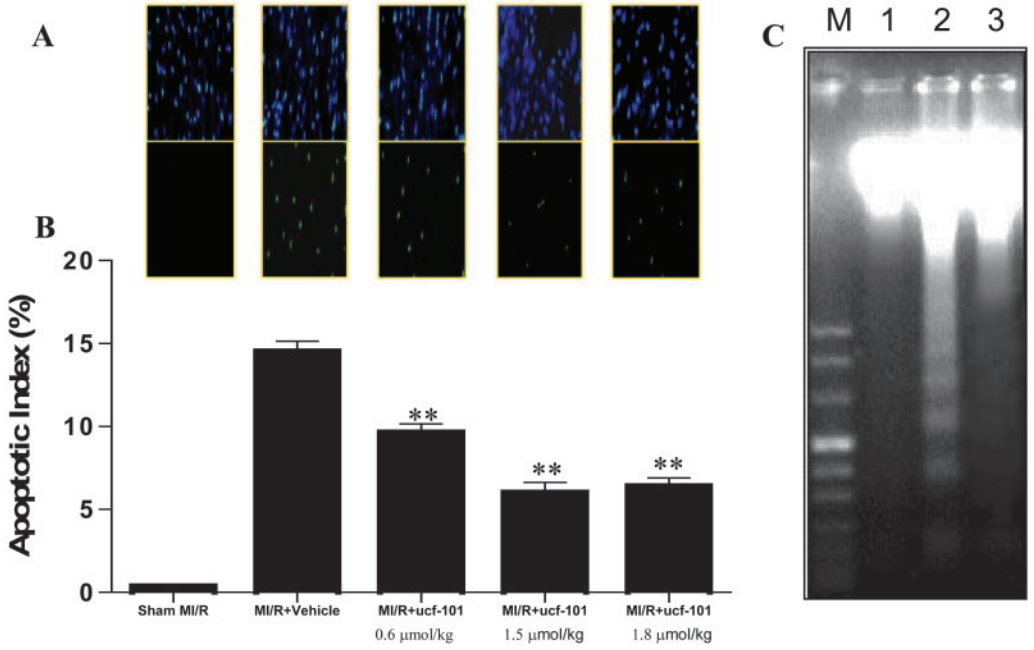


**Figure 2.** Dose-response relation of ucf-101 on postischemic myocardial apoptosis as determined by TUNEL staining (n=8 to 10 animals per group). Cardiac myocytes were identified by anti–α-actinin antibody, total nuclei were labeled with 4′6-diamidine-2-phenylindole, and apoptotic nuclei were detected by green fluorescein staining (A). For each slide, 10 fields were randomly chosen and 8 slides were examined for each heart (B). Assays were performed in blinded manner. $^{**}P<0.01$ vs vehicle. C, Representative gel picture (from 5 independent experiments) of in situ detection of DNA fragments in heart tissue from mice subjected to sham ischemia or 30 minutes of ischemia followed by 3 hours of reperfusion receiving vehicle or ucf-101 (1.5 μmol/kg). M indicates molecular marker; 1, sham MI/R; 2, MI/R+vehicle; and 3, MI/R+ucf-101. All other abbreviations are as defined in text.

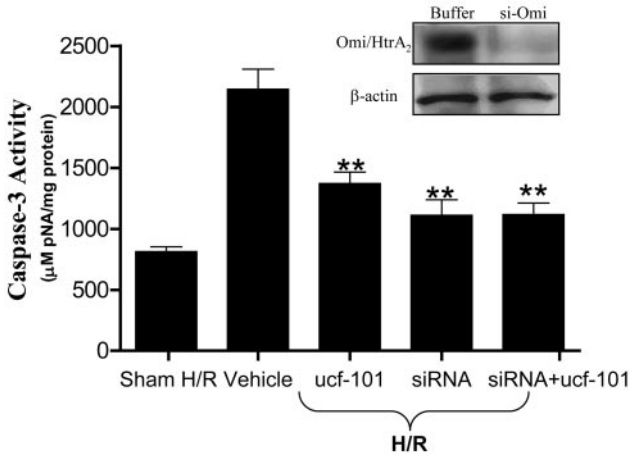


**Figure 3.** Suppression of Omi/HtrA2 by RNA interference decreases hypoxia (H)/(R) reoxygenation–induced cardiomyocyte apoptosis. Insert, Western blot showing that transfecting cells with siRNA molecules against Omi effectively eliminated Omi/HtrA2 protein expression. All other abbreviations are as defined in text. $^{**}P<0.01$ vs vehicle.

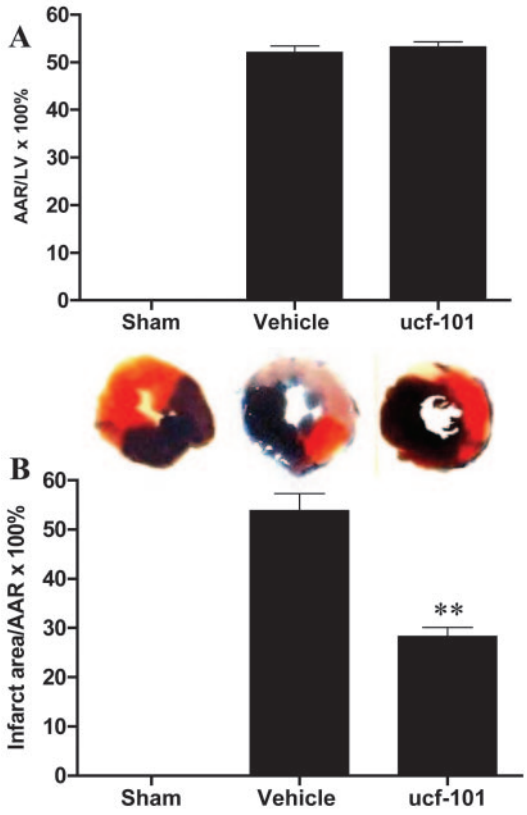


**Figure 4.** Effect of ucf-101 on myocardial infarct size after 3 hours of ischemia followed by 24 hours of reperfusion (n=10 to 12 animals per group). A, Percentage of AAR, as identified by negative Evans blue staining, divided by total left ventricular (LV) area. B, Percentage of infarct area (as identified by negative TTC staining) divided by AAR. ** $P<0.01$ vs MI/R+vehicle. All other abbreviations are as defined in text.

significant inhibition of apoptosis, the effect of ucf-101 on DNA ladder formation, a hallmark of apoptotic cell death, was determined. As illustrated in Figure 2C, 30 minutes of ischemia followed by 3 hours of reperfusion resulted in significant DNA fragmentation, which was significantly reduced by treatment with ucf-101 before reperfusion. These results demonstrate that Omi/HtrA2, after it translocates from the mitochondria to the cytosol, contributes to postischemic myocardial apoptosis.

Although our previous study had demonstrated that ucf-101 is highly selective to Omi/HtrA2,[13] it was possible that ucf-101 might have additional unrecognized effects that were responsible for its observed antiapoptotic effect. To provide a definitive assessment of the proapoptotic effect of Omi/HtrA2 in myocardial apoptosis and to further demonstrate that ucf-101 reduces myocardial apoptosis by inhibiting Omi/HtrA2 activity, an additional in vitro experiment (in rat neonatal ventricular cardiomyocytes) with the use of small interference RNA (siRNA) was performed. The cardiomyocytes were transfected twice in a 24-hour interval with siRNA (si-Omi sense, 5′-GGGGAGUUUGUUGUUGCCAdTdT-3′ and si-Omi antisense, 5′UGGCAACAACAAACUCC-CCdTdT-3′, Dharmacon) with the use of Oligofectamine reagent (Invitrogen) according to the manufacturer's instructions. This double-transfection method has been shown to markedly increase transfection efficiency and to eliminate Omi/HtrA2 expression.[18] Twenty-four hours after the second transfection, cells were either lysed for analysis of Omi/HtrA2 expression by Western blotting or subjected to hypoxia (8 hours) and reoxygenation (2 hours) as described in our previous study.[19] As illustrated in Figure 3, transfecting cells with siRNA molecules against Omi effectively eliminated Omi/HtrA2 protein expression. Most important, this treatment markedly reduced hypoxia/reoxygenation–induced cardiomyocyte apoptosis as assessed by caspase-3 activity (Figure 3). Moreover, although treatment with ucf-101 significantly reduced myocardial apoptosis in nontransfected cells, the addition of ucf-101 failed to exert any additional protective effect in transfected cells (Figure 3). These results provide strong evidence that Omi/HtrA2 plays a critical role in myocardial apoptosis and that ucf-101 exerted its cardioprotection by inhibiting Omi/HtrA2 activity.

To determine whether the cardioprotective effects of ucf-101 were sustained, an additional group of animals was subjected to 30 minutes of ischemia and 24 hours of reperfusion, and the effect of ucf-101 on myocardial infarct size was determined. As summarized in Figure 4A, there was no difference in the value of AAR/left ventricle between vehicle-treated and ucf-101–treated animals, indicating that a comparable degree of ischemia was induced in both groups. However, treatment with ucf-101 significantly reduced myocardial infarct size ($P<0.01$, Figure 4B).

## Mechanisms by Which Omi/HtrA2 Promotes Myocardial Apoptosis After I/R

A recent in vitro study demonstrated that Omi/HtrA2 enhances apoptotic cell death by both premitochondrial (enhancing mitochondrial permeability) and postmitochondrial (inhibiting and or/degrading XIAP) mechanisms.[20] To determine whether cytosolic Omi/HtrA2 in I/R cardiac tissue may also increase I/R-induced myocardial apoptosis by a premitochondrial mechanism, we determined the effect of ucf-101

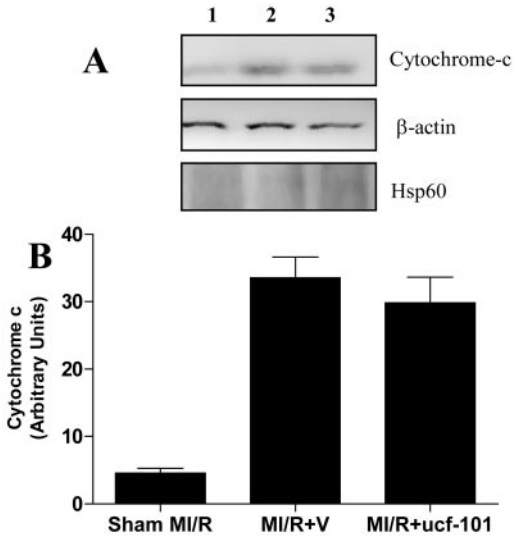


**Figure 5.** Effect of ucf-101 on mitochondrial cytochrome *c* release in I/R myocardial tissue. A, Representative Western blot of cytosolic cytochrome *c*. Lane 1, sham MI/R; lane 2, MI/R+vehicle (V); lane 3, MI/R+ucf-101. Hsp indicates heat shock protein. B, Densitometry analysis of Western blot of cytosolic cytochrome *c* (n=5 or 6 animals per group). Bar heights represent mean values and brackets indicate SE. All other abbreviations are as defined in text.

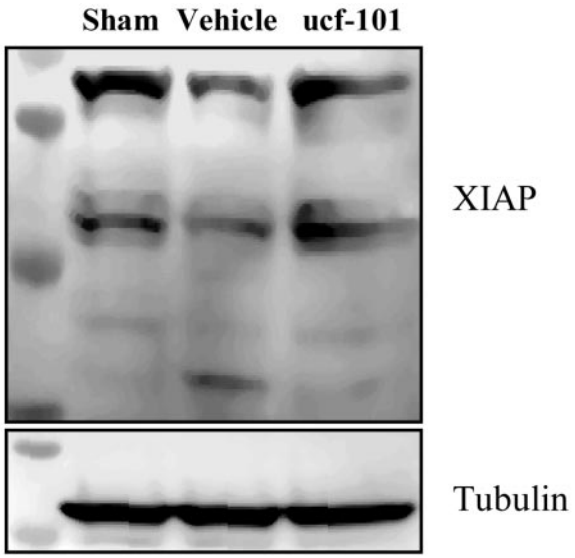


**Figure 7.** Representative Western blots (from at least 5 independent experiments per group) show that MI/R resulted in XIAP degradation, which was partially blocked by ucf-101.

on cytochrome *c* release. As summarized in Figure 5, MI/R resulted in an 8-fold increase in cytosolic cytochrome *c* level. However, treatment with ucf-101 failed to block I/R-induced cytochrome *c* release. This result suggests that it is unlikely that Omi/HtrA2 exerts its proapoptotic effect through mechanisms at a premitochondrial level.

Cilenti and colleagues[13] recently reported that treatment with ucf-101 markedly inhibited apoptotic cell death in caspase-9–null fibroblasts and concluded that Omi/HtrA2 may cause apoptotic cell death in a caspase-independent fashion via its proteolytic activity. To determine whether cytosolic Omi/HtrA2 may increase I/R cardiomyocyte apoptosis in a comparable fashion, we measured I/R-induced caspase-9 and caspase-3 activation and determined whether ucf-101 had any effect on I/R-induced caspase activation. As expected, both caspase-9 and caspase-3 activities were markedly increased in I/R cardiac tissue. To our surprise, treatment with ucf-101, a compound that does not have any direct inhibitory effect on caspases,[13] significantly reduced caspase-9 and caspase-3 activity in I/R hearts (Figure 6). This result strongly suggests that in I/R cardiomyocytes, Omi/HtrA2 increases apoptosis via proteolytic degradation of proteins that normally inhibit caspase activity.

Two recent studies have independently demonstrated that unlike Smac/DIABLO, Omi/HtrA2 not only directly binds to XIAP through its reaper motif but also results in XIAP degradation through its protease activity.[18,21] To investigate whether Omi/HtrA2 may increase apoptosis in I/R cardiomyocytes by degradation of XIAP, we determined XIAP levels in I/R hearts. As illustrated in Figure 7, 30 minutes of ischemia followed by 3 hours of reperfusion resulted in significant XIAP degradation, which was markedly inhibited by treatment with ucf-101. These results provide direct

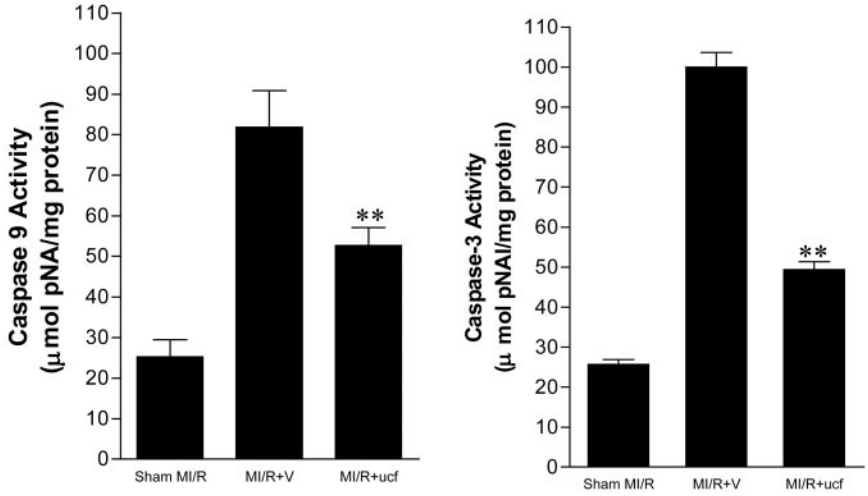


**Figure 6.** Effect of ucf-101 on caspase-9 (left) and caspase-3 (right) activity in I/R myocardial tissue. Caspase activity was determined by using caspase colorimetric assay kits and following manufacturer's instructions. ** $P<0.01$ vs MI/R+vehicle (V). n=9 to 12 mice per group.

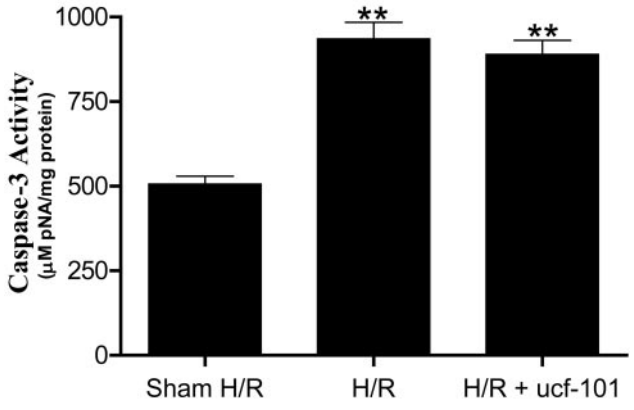


**Figure 8.** Treatment with ucf-101 failed to reduce hypoxia (H)/reoxygenation (R)–induced caspase-3 activation in mnd2 mouse embryonic fibroblasts. $^{**}P<0.01$ vs sham H/R.

evidence that Omi/HtrA2 likely promotes apoptosis in I/R hearts by its protease activity that results in XIAP degradation and subsequent caspase activation.

To provide additional evidence that ucf-101 preserved XIAP activity by inhibiting the protease activity of Omi/HtrA2, an additional study in mouse embryonic fibroblasts isolated at E15 from mnd2/mnd2 embryos was performed. A previous study had demonstrated that the mnd2 mouse contains a naturally occurring missense mutation in the protease domain of Omi/HtrA2.[22] The mutation does not affect the processing or localization of the mutant Omi protein but renders it proteolytically inactive. As summarized in Figure 8, treatment with ucf-101 had no effect on hypoxia/reoxygenation-induced caspase-3 activation in mnd2 mouse embryonic fibroblasts. These results further suggest that ucf-101 reduces myocardial apoptosis by inhibiting the protease activity of Omi/HtrA2 rather than by inhibiting Omi/HtrA2-XIAP binding.

## Discussion

We have demonstrated for the first time that Omi/HtrA2 is translocated from the mitochondria to the cytosol in cardiomyocytes after I/R. Using a novel, selective Omi/HtrA2 inhibitor, we have provided direct evidence that mitochondrial release of Omi/HtrA2 contributes to postischemic myocardial apoptosis and that blockade of Omi/HtrA2's protease activity not only reduced postischemic myocardial apoptosis but also decreased myocardial infarct size after a prolonged reperfusion period. Although considerable evidence from in vitro studies suggests that overexpression of Omi/HtrA2 promotes apoptosis, the role of Omi/HtrA2 in a real pathological condition had not been previously reported. In addition, we have demonstrated that in I/R cardiomyocytes, Omi/HtrA2 increases apoptosis through a protease activity–dependent, caspase-mediated mechanism, which involves degradation of a critical antiapoptotic molecule, XIAP.

IAPs were first identified in baculoviruses as proteins able to protect insect cells from apoptosis. Since then, several mammalian IAPs have been identified. All IAPs contain one or more conserved domains, referred to as baculovirus IAP repeats (BIRs), that are essential for inhibition of apoptosis.[6] The BIR domains and the linker regions between them bind directly to caspases and inhibit their activity. The antiapoptotic activity of IAPs is regulated by a group of proteins that bind to the BIR domains of IAPs through an *N*-terminal conserved, 4-residue, IAP-binding motif (IBM).[5] In *Drosophila melanogaster*, 5 IBM-containing proteins known as Reaper, Hid, Grim, Sickle, and Jafrac2 have been identified as direct IAP-binding proteins. These proteins promote caspase activation by disrupting caspase-IAP complexes and/or inducing autoubiquitination and degradation of IAPs, thus preventing IAPs from inhibiting caspases. In mammals, 2 functional homologues of the *Drosophila* proteins, known as Smac/DIABLO and Omi/HtrA2, have been identified.[12,23] Both Smac/DIABLO and Omi/HtrA2 are synthesized as precursor proteins with *N*-terminal mitochondrial localization signal peptides that are removed during maturation in the mitochondria to expose their *N*-terminal IBM. During apoptosis, both proteins are released from the intermembrane space of the mitochondria into the cytoplasm and promote caspase activation and apoptosis by binding to the BIR domain(s) of XIAP. Both Smac/DIABLO and Omi/HtrA2 release has been shown to be promoted by the Bcl2-homology 3-only Bcl-2 family member truncated Bc12-homology 3–interacting domain (tBid).[24] This has been recently confirmed in an in vivo model, demonstrating that intravenous injection of agonistic anti-Fas antibody results in mitochondrial release of Omi/HtrA2 in a tBid-dependent fashion in liver cells.[11]

Although Omi/HtrA2 and Smac/DIABLO both seem to target XIAP once released into the cytosol, increasing evidence suggests that Omi/HtrA2 may play a unique role in apoptosis. Several different Smac/DIABLO-deficient cells respond normally to various apoptotic stimuli, suggesting the existence of a redundant molecule or molecules compensating for a loss of Smac/DIABLO function.[25] In contrast, Omi/HtrA2-knockdown cells have shown to be more resistant to apoptotic stimuli.[8,9] In addition, several recent studies have demonstrated that overexpression of Omi/HtrA2 markedly increases apoptosis.[9,13,26] These data suggest a nonredundant essential function of Omi/HtrA2 in the induction of apoptosis. However, it should be noted that just because overexpression of HtrA2 in the cytosol can promote cell death does not mean that this is its role in vivo. Overexpression of other active serine proteases, such as proteinase K, also results in apoptosis,[27] but induction of cell death by this protease is clearly unrelated to its physiological function. In the present study, we used a novel, selective Omi/HtrA2 inhibitor and demonstrated that inhibition of Omi/HtrA2 markedly reduced myocardial apoptosis after I/R. This result provides novel, direct evidence that a normal level of an endogenously expressed Omi/HtrA2 contributes to apoptosis under a real pathological condition, ie, in vivo myocardial ischemia followed by reperfusion.

Unlike Smac/DIABLO, the proapoptotic activity of Omi/HtrA2 involves both IAP binding and serine protease activity. Mutations of either the *N*-terminal alanine of mature Omi/HtrA2, essential for IAP interaction, or the catalytic serine residue reduces the ability of HtrA2 to promote cell death, whereas a complete loss of proapoptotic activity is observed

when both sites are mutated.[7] However, 2 essential questions remain for determining the mechanisms by which Omi/HtrA2 stimulates apoptosis. First, the relative contribution of IAP binding versus serine protease activity of Omi/HtrA2 to its proapoptotic effect remains undetermined, and this is likely to be cell type and stimulation specific. Second, the specific substrate whose degradation and inactivation by Omi/HtrA2 promotes apoptosis has not been identified. In a recent study, Suzuki et al[20] reported that extramitochondrial expression of Omi/HtrA2 causes permeabilization of the outer mitochondrial membrane, leading to cytochrome *c* release. They proposed that Omi/HtrA2 may cause degradation of proteins that regulate apoptosis at a premitochondrial level. However, our present results demonstrated that treatment with ucf-101 failed to decrease cytochrome *c* release in I/R hearts and thus, argue against Omi/HtrA2's promoting cardiomyocyte apoptosis at a premitochondrial level. Recent studies have demonstrated that Omi/HtrA2 binding to IAP results in its catalytic degradation, causing irreversible inactivation of IAPs.[18,21] We have provided the first in vivo evidence that significant XIAP degradation occurring in I/R hearts was markedly attenuated by ucf-101 treatment. This suggests that in I/R cardiomyocytes, XIAP is degraded by cytosolic Omi/HtrA2 and thus, causes the loss of its anticaspase effect.

In summary, we have demonstrated that I/R resulted in Omi/HtrA2 translocation from the mitochondria to the cytosol. Once released, cytosolic Omi/HtrA2 caused XIAP degradation, caspase activation, and subsequent apoptosis. Therapeutic interventions that inhibit Omi/HtrA2 expression, translocation, or protease activity (such as the ucf-101 inhibitor) may provide an effective method in the treatment of cardiovascular diseases in which apoptotic cell death plays an important role.



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