Differential effects of caspase inhibitors on endotoxin-induced myocardial dysfunction and heart apoptosis

HAROLD FAUVEL, PHILIPPE MARCHETTI, CLAUDE CHOPIN, PIERRE FORMSTECHER, AND REMI NEVIERE. Differential effects of caspase inhibitors on endotoxin-induced myocardial dysfunction and heart apoptosis. Am J Physiol Heart Circ Physiol 280: H1608–H1614, 2001.—Endotoxin is one of the major factors causing myocardial depression and death during sepsis in humans. Recently, it was reported that endotoxin may induce cardiomyocyte apoptosis. Also, multiple caspase activation has been implicated in endotoxin-induced apoptosis in several organ systems. In this study, we investigated whether endotoxin would increase myocardial caspase activities and evaluated the effects of in vivo administration (3 mg/kg) of the broad-spectrum caspase inhibitor benzoyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (z-VAD.fmk), the caspase-3-like inhibitor benzoyloxycarbonyl-Asp-Glu-Val-Asp-chloromethylketone (z-DEVD.cmk), and the caspase-1-like inhibitor acetyl-Tyr-Val-Ala-Asp-chloromethylketone (Ac-YVAD.fmk), on endotoxin-induced myocardial dysfunction and apoptosis. Endotoxin administration (10 mg/kg iv) induced myocardial contractile dysfunction that was associated with caspase activity increases and nuclear apoptosis. Broad-spectrum z-VAD.fmk and z-DEVD.cmk improved endotoxin-induced myocardial dysfunction and reduced caspase activation and nuclear apoptosis when given immediately and 2 h after endotoxin. In contrast, no effects of Ac-YVAD.fmk were observed on myocardial function and caspase-induced apoptosis. Administration of caspase inhibitors 4 h after endotoxin treatment was not able to protect the rat heart from myocardial dysfunction and nuclear apoptosis. These observations provide evidence that in our model, caspase activation plays a role in endotoxin-induced myocardial apoptosis. Caspase inhibition strategy may represent a therapeutic approach to endotoxin-induced myocardial dysfunction.

SEPTIC SHOCK IS A MAJOR CAUSE of morbidity and mortality commonly observed in patients with septic shock. Myocardial depression can be demonstrated in experimental animal models following the administration of Escherichia coli endotoxin or lipopolysaccharide, the major toxin of gram-negative bacteria. In this context, a number of components of the host septic inflammatory cascade response have been shown to contribute to ventricular dysfunction, including myocardial microvascular abnormalities (3), the presence of activated leukocytes (2), and the effects of circulating and locally produced proinflammatory cytokines (tumor necrosis factor-α) on the heart (17). Moreover, recent information indicates myocardial cell injuries may be involved in human septic shock (22).

Recently, we (13) and others (8) demonstrated that endotoxin in vivo may induce rat cardiomyocyte apoptosis and cardiac dysfunction. Indeed, relevant levels of tumor necrosis factor-α may induce apoptosis of cardiomyocytes in vitro (7). In most cases, the initiation and execution phases of the apoptotic process involve activation of a family of aspartate-specific cysteine proteases called caspases. Caspases can be divided on the basis of the substrate specificities and also into functional subfamilies (15). Group I enzymes including caspases-1, -4, -5, and -13 mediate cytokine maturation and inflammation. The apoptotic caspases (groups II and III) are involved in a hierarchically ordering proteolytic cascade. Group III activators (caspases -8, -6, -9, -10) act upstream of group II effector caspases (caspases-3, -7, -2) that are responsible for the cleavage of crucial substrates in the final degradation phase of the apoptotic cell death. Caspase-3 activity, which leads to nuclear apoptosis, has been extensively involved in human pathologies such as dilated cardiomyopathies, terminal heart failure, and ischemia reperfusion injury (12, 23). Moreover, we have shown that caspase-1, -3, -8, and -9 activities were increased in hearts 4 h after endotoxin challenge (13). Importantly, pharmacological inhibition of multiple caspases with the broad-spectrum caspase inhibitor z-VAD.fmk im-

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proves both myocardial function and reduces apoptotic cell death in various experimental models including myocardial ischemia (23).

In this study, we tested whether endotoxin administration would increase myocardial multiple caspase activities and apoptosis and whether different caspase inhibitors would reduce endotoxin-induced myocardial dysfunction and apoptosis. Therefore, the specific objectives of the present study were to determine the effects of endotoxin on caspase activities and nuclear apoptosis and to examine the effects of in vivo administration of caspase inhibitors on endotoxin-induced myocardial dysfunction, caspase activation, and nuclear apoptosis. First, we evaluated the effects of endotoxin administration on myocardial function and biochemical parameters, including caspase-1, -3, and -8 activities and DNA fragmentation. Second, we evaluated the effects of peptide-based inhibitors of caspases [a broad-spectrum caspase inhibitor benzoylcarbonyl-Val-Ala-Asp-fluoromethylketone (z-VAD.fmkk), a caspase-3-like inhibitor benzoylcarbonyl-Asp-Glu-Val-Asp-chloromethylketone (z-DEVD.cmkk), and a caspase-1-like inhibitor acetyl-Tyr-Val-Ala-Asp-chloromethylketone (Ac-YVAD.fmkk)] on myocardial function, multiple caspase activities, and nuclear apoptosis in the hearts from endotoxin-treated rats when given immediately, 2, and 4 h after endotoxin infusion. Overall, our observations suggest that, in our model, caspase activation plays an important role in the pathophysiology of endotoxin-induced myocardial dysfunction.

MATERIALS AND METHODS

Animal preparation. Adult male Sprague-Dawley rats (250–300 g) (Dépré; Saint Doullard, France) were housed for 6 days in groups of six in standard cages and supplied ad libitum with laboratory chow and tap water. Treatments were administered intravenously via the dorsal penine vein after brief ether anesthesia. Overall, eight groups of rats were administered intravenously via the dorsal penine vein ad libitum with laboratory chow and tap water. Treatments were conducted in accordance with our institution’s guidelines for the care and use of laboratory animals.

Histological studies. After the rats were euthanized by pentobarbital overdose, the hearts were excised and placed in ice-cold Krebs-Henseleit buffer (KHB) solution containing (in mM) 118 NaCl, 4.75 KCl, 1.19 KH2PO4, 1.19 MgSO4, 2.54 CaCl2, 25 NaHCO3, 0.5 EDTA, and 11 glucose; and immediately resuspended with ice-cold lysis buffer (50 mM HEPES, pH 7.4, 0.1% 3-[3-cholamidopropyl]dimethylammonio)-1-propanesulfonate, 5 mM dithiothreitol, 0.1 mM EDTA) containing 1 mM phenylmethylsulfonyl fluoride and 10 μg/ml aprotinin and leupeptin agitated 45 min. Tissues were homogenized and then centrifuged at 14,000 g for 10 min and the supernatants were used. Proteins (200 μg) were diluted with assay buffer (50 mM HEPES, pH 7.4, 100 mM NaCl, 0.1% 3-[3-cholamidopropyl]dimethylammonio)-1-propanesulfonate, 10 mM dithiothreitol, 2 mM EDTA, 10% glyceral, 1 mM phenylmethylsulfonyl fluoride) and incubated at 25°C with the colorimetric substrates (Biomol; Plymouth Meeting, PA) Ac-DEVD-pNA (200 μM), Ac-YVAD-pNA (200 μM), or Ac-IETD-pNA (200 μM) in 96-well microtiter plates. Cleavage of the p-nitroaniline (p-NA) dye from the peptide substrate was determined by measuring the absorbance of p-NA at 405 nm in a microplate reader (Digiscan, Asys Hitech; Cincinnati, OH). Results were calibrated with known concentrations of p-NA and expressed in picomoles of substrate cleaved per minute and per microgram of protein at 25°C.

DNA fragmentation detection. For the detection of oligonucleosomes, a Cell Death Detection ELISAPLUS kit (Roche) was used according to manufacturer instructions. Small pieces (40–50 mg) of hearts were homogenized in the provided lysis buffer for 45 min at room temperature, followed by centrifugation for 10 min at 2,000 rpm. The protein level of the supernatants was determined. Supernatant (20 μl) was subjected immediately to the ELISA test. For electrophoresis, DNA was extracted from cardiac tissue (LV apex) using a commercially available isolation kit (Genzyme TACS, R&D Systems; Minneapolis, MN). The DNA obtained was used in a ligation-mediated polymerase chain reaction assay according to manufacturer instructions (Clontech Laboratories; Palo Alto, CA). After 25 cycles, DNA electrophoraphoresis (10 ng/lane) was run through 1.2% agarose-ethidium bromide gel at 6 V/cm for 4 h.

Isolated and perfused heart preparation. Myocardial contractile function was studied using a modified Langendorff isolated heart preparation as previously described (14). After heparinization and ether anesthesia, the hearts were rapidly excised and placed in ice-cold KHB solution. The hearts were...
then mounted onto a Langendorff heart perfusion apparatus and perfused in a retrograde fashion via the aorta at a constant flow rate of 10 ml/min with aerated (95% O2-5% CO2) KHB at 37°C. Cardiac contractile function was assessed using a water-filled latex balloon inserted in the LV cavity and connected to a pressure transducer. This balloon was then adjusted to a LV end-diastolic pressure (LVEDP) of 5 mmHg. The hearts were paced at 300 beats/min and allowed to equilibrate for 30 min. LV developed pressure (LVDP), its maximum and minimum first derivatives (dP/dt max and dP/ d t min), and coronary perfusion pressure (CPP) were monitored and recorded on a chart recorder (Kontron; Basel, Switzerland). After baseline measurements, the LVDP and LV preload relationship (LVEDP) was set to 5 mmHg, and hearts were paced at 300 beats/min and allowed to equilibrate for 30 min. LV developed pressure (LVDP), its maximum and minimum first derivatives (dP/dt max and dP/ d t min), and coronary perfusion pressure (CPP) were monitored and recorded on a chart recorder (Kontron; Basel, Switzerland). After baseline measurements, the LVDP and LV preload relationship (LVEDP) was set to 5 mmHg, and hearts were paced at 300 beats/min and allowed to equilibrate for 30 min before measurements were made. LVDP, left ventricular developed pressure; CPP, coronary perfusion pressure; dP/dt max, maximum rate of LV pressure rise; dP/dt min, maximum rate of LV pressure fall; CPP, coronary perfusion pressure. *P < 0.05 for Dunnett post hoc test comparing with control group (sham-treated animals). Statistical significance was assigned to P < 0.05.

**RESULTS**

*Effects of endotoxin administration on myocardial function, multiple caspase activity, and nuclear apoptosis.* Isolated heart function studies revealed that myocardial performance was progressively reduced from 4 to 14 h after in vivo endotoxin challenge (Table 1). In the same model, we studied the time-course analysis of caspase activities (caspase-1, -3, and -8-like activities) and nuclear apoptosis after endotoxin injection (Fig. 1). Caspase -1, -3, and -8-like activities were increased from 2 h after endotoxin infusion (Fig. 1A). The highest level of caspase-3 and -8 activities was reached from 4 h after endotoxin treatment, whereas YVADase activity reached a peak 2 h after endotoxin treatment (Fig. 1A). As shown in Fig. 1B, significant nuclear fragmentation detected by agarose gel electrophoresis was first evident at 4, 8, and 14 h after endotoxin injection. The TUNEL method confirmed that cardiomyocytes presented nuclear apoptosis 8 h after endotoxin injection (Fig. 2).

*Effects of caspase inhibitors on endotoxin-induced myocardial dysfunction and apoptosis.* We examined the influence of caspase inhibition on myocardial function and apoptosis-related parameters 8 h after endotoxin administration. This time allowed us to evaluate the effects of caspase inhibitors when administered immediately, 2, and 4 h after endotoxin infusion. Compared with sham-treated animals, 8 h after endotoxin injection, isolated and perfused hearts from endotoxin-treated animals displayed a shift downward of the LVDP-preload relationship curves, in the direction of reduced LV systolic performance (Fig. 3A). Compared with sham animals, heart caspase-3 activity (n = 6 hearts in each group) and heart lysate oligonucleosome formation (n = 9 hearts in each group; Fig. 3, B and C) were increased in endotoxin-treated animals.

**Table 1. Assessment of cardiac function after endotoxin injection**

<table>
<thead>
<tr>
<th></th>
<th>0 h</th>
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<th>4 h</th>
<th>8 h</th>
<th>14 h</th>
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<tr>
<td>LVDP, mmHg</td>
<td>89 ± 5</td>
<td>83 ± 6</td>
<td>50 ± 7*</td>
<td>46 ± 5*</td>
<td>57 ± 9*</td>
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<tr>
<td>LVEDP, mmHg</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>dP/dt max, mmHg/s</td>
<td>4,360 ± 212</td>
<td>4,010 ± 260</td>
<td>2,440 ± 300*</td>
<td>2,080 ± 250*</td>
<td>2,990 ± 435*</td>
</tr>
<tr>
<td>dP/dt min, mmHg/s</td>
<td>3,120 ± 140</td>
<td>2,800 ± 275</td>
<td>1,740 ± 200*</td>
<td>1,385 ± 165*</td>
<td>2,180 ± 290*</td>
</tr>
<tr>
<td>CPP, mmHg</td>
<td>39 ± 4</td>
<td>39 ± 2</td>
<td>44 ± 9</td>
<td>42 ± 8</td>
<td>39 ± 7</td>
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</table>

Results are means ± SE (n = 6 in each group). Comparisons between means were made using one-way ANOVA. Endotoxin (10 mg/kg, iv)-treated rats were studied immediately (0 h), 2, 4, 8, and 14 h after endotoxin administration. Left ventricular end-diastolic pressure (LVEDP) was set to 5 mmHg, and hearts were paced at 300 beats/min and allowed to equilibrate for 30 min before measurements were made. LVDP, left ventricular developed pressure; CPP, coronary perfusion pressure; dP/dt max, maximum rate of LV pressure rise; dP/dt min, maximum rate of LV pressure fall; CPP, coronary perfusion pressure. *P < 0.05 for Dunnett post hoc test comparing with endo (0 h; immediately after endotoxin infusion). See MATERIALS and METHODS for treatment group design.
The broad-spectrum caspase inhibitor z-VAD.fmK reduced endotoxin-induced heart dysfunction (Fig. 3A) and was also able to reduce endotoxin-induced heart lysate oligonucleosome formation \( (n = 9 \text{ hearts in each group}) \) and caspase-3 activity \( (n = 6 \text{ hearts in each group}) \) increases when given immediately (Fig. 3, B and C) and 2 h after endotoxin challenge (Fig. 4, A–C). These protective effects were not observed when z-VAD.fmK was administered 4 h after endotoxin treatment challenge (Fig. 4, A–C).

The caspase-3-like activity inhibitor z-DEVD.cmK reduced endotoxin-induced heart dysfunction (Fig. 3A) and was also able to reduce endotoxin-induced heart lysate oligonucleosome formation \( (n = 9 \text{ hearts in each group}) \) and caspase-3 activity \( (n = 6 \text{ hearts in each group}) \) increases when given immediately (Fig. 3, B and C) and 2 h after endotoxin challenge (Fig. 4, A–C). These protective effects were not observed when z-DEVD.cmK was administered 4 h after endotoxin treatment challenge (Fig. 4, A–C).

The caspase-1-like inhibitor Ac-YVAD.cmK was totally unable to protect against endotoxin-induced heart dysfunction and to alter heart lysate oligonucleosome formation and caspase-3 activity increases in endotoxin-treated animals when given immediately (Fig. 3, A–C), 2, and 4 h after endotoxin challenge (Fig. 4, A–C).

**DISCUSSION**

Caspase activation is a critical process leading to apoptotic cell death. We hypothesized that caspase activation and caspase inhibition would have an important role in sepsis-induced myocardial dysfunction and nuclear apoptosis. In our model of sepsis, we found that endotoxin administration induced a severe and sustained reduction of LV systolic performance. Endotoxin-induced myocardial dysfunction was timely associated with multiple caspase activation and nuclear apoptosis. Broad-spectrum and effector caspase (caspase-3) inhibitors reduced myocardial dysfunction, caspase-3 activity, and nuclear apoptosis when given immediately and even 2 h after endotoxin administration. In contrast, the caspase-1 inhibitor had no effect on these parameters. Hence, this study provides the following new information: 1) caspase-3 activation and nuclear DNA fragmentation are related in a timely manner to endotoxin-induced myocardial dysfunction.

![Fig. 1. Time-course apparition of caspase activities and nuclear apoptosis in hearts from rats treated with endotoxin. A: determination of multiple caspase activities in hearts from rats treated with endotoxin. Caspase-1-like activity, caspase-3-like activity, and caspase-8-like activity corresponding to Thr-Val-Ala-Aspase (YVADase), Asp-Glu-Val-Aspase (DEVDase), and Ile-Glu-Thr-Aspase (IETDase) enzymatic activities, respectively, were measured at indicated times after endotoxin challenge with specific \( p \)-nitroaniline (\( p \)-NA)-substrates as described in MATERIALS AND METHODS. Results are expressed as picomoles of substrate \( p \)-NA hydrolyzed per minute and per microgram of proteins. Data (means ± SE) of a representative experiment (n = 8 hearts in each group) made in duplicate. Compared with baseline (0 h immediately after endotoxin injection), YVADase, DEVDase, and IETDase enzymatic activities were increased at 2, 4, 8, and 14 h after endotoxin challenge (one-way ANOVA; \( \ast P < 0.05 \) for Dunnett post hoc test compared with baseline, 0 h). B: analysis of nuclear fragmentation by agarose gel electrophoresis. Time-course apparition of endotoxin-induced nucleosomal ladders were visualized after ligation-mediated polymerase chain reaction assay (MATERIALS AND METHODS). Results are representative for three independent experiments.](http://ajpheart.physiology.org/)

![Fig. 2. In situ determination of nuclear apoptosis in hearts 8 h after endotoxin (Endo) injection. In situ analysis of myocardial samples stained with transferase-mediated dUTP nick end labeling (TUNEL) peroxidase staining. Eight hours after treatment 4-\( \mu \)m paraffin-embedded tissue sections were prepared from the left ventricular (LV) apex obtained from sham rats (n = 5 tissue sections; left) or endotoxin-treated rats (n = 5 tissue sections; right). Original magnification \( \times 400 \). Arrow indicates TUNEL-positive nucleus.](http://ajpheart.physiology.org/)
and 2) treatment with effector caspase inhibitors ameliorates endotoxin-induced myocardial dysfunction and reduces caspase-induced nuclear apoptosis.

Recently, apoptosis has been implicated in the pathophysiology of human cardiovascular diseases, including dilated cardiomyopathy, myocarditis, heart failure, and ischemic heart disease (for review, see Ref. 5). Apoptosis has been extensively described as a determinant process in sepsis-associated cell death of different cell types, including hepatocytes (6), enterocytes (16), or endothelial cells (9), but limited observations have been reported in myocardial tissue (11). Here, we provided strong evidence for the involvement of apoptosis in hearts from endotoxin-treated rats by using numerous criteria such as oligonucleosomal DNA fragmentation and activity of multiple caspases. In our model, endotoxin administration induced progressive reduction in LV systolic performance, which was maximal 8 h after endotoxin challenge. We found that endotoxin administration induced a time-dependent increase in caspase-8- and caspase-3-like activities. In contrast, the activity of caspase-1, representing the prototypic proinflammatory caspase, was slightly increased in our septic model. Consistently, activation of caspases-2, -3, -6, and -9, but not caspase-1, has been also demonstrated in thymocyte apoptosis in a clinically relevant model of sepsis (20). Furthermore, endotoxin has been proven to induce only moderate caspase-1 activity in freshly isolated peripheral blood monocytes (19). The pivotal role of caspases in our septic heart model was further demonstrated by the fact that injection of the broad-spectrum caspase inhibitor, z-VAD.fmk not only inhibited the activation of caspases and nuclear apoptosis but also endotoxin-induced myocardial dysfunction (Fig. 3). Interestingly, it has been reported (4) that novel peptidomimetic fluoromethylketone, which inhibits numerous caspases and apoptosis, also rescues mice from lethal endotoxin shock. To further evaluate the incidence of proinflammatory caspase-1 and apoptotic effector caspase-3, we used in vivo inhibitors with the Tyr-Val-Ala-Asp and Asp-Glu-Val-Asp motifs described as preferential inhibitors of the caspase-1 and caspase-3 subfamilies, respectively. The peptide-based caspase inhibitors used were the halomethyl ketone inhibitors, which have a broad-spectrum of activity and may potently inhibit multiple caspases. However, the inhibitory constants of synthetic caspase inhibitors Ac-YVAD.cmk and z-DEVD.cmk (8) were 0.3 and 0.7 µM/s for caspase-1 and caspase-3, respectively, suggesting potential preferential inhibition.

In our experimental model, caspase-1 inhibitor (Ac-YVAD.cmk), although it inhibits YVADase activity (data not shown), had no effect on endotoxin-induced myocardial dysfunction, downstream caspase-3 activity increase, and nuclear apoptosis. Caspase-1, formerly known as interleukin-1β-converting enzyme, participates in proteolytic processing of several cytokines (for review, see Ref. 18). In our model of endo-

**Fig. 3.** Effects of coinjection of caspase inhibitors on endotoxin-induced heart alterations. A: changes of LV systolic performance in rat hearts 8 h after injection with endotoxin alone or with caspase inhibitors (n = 6 hearts in each group). Results are representative of the means ± SE. LV end-diastolic pressure (LVEDP) was increased incrementally from -5 to +20 mmHg to construct LV developed pressure (LVPD)-preload relationship curves. Statistical comparisons between means were made by two-way ANOVA for repeated measurements on preload: treatment (five levels) × preload pressure (six levels). Post hoc analyses were made using the Dunnett test comparing variable group with control group (sham-treated animals). Compared with sham rats, LVPD-preload relationships were shifted downward (in the direction of LV systolic performance decrease) in endotoxin-treated rats and in endotoxin + Ac-YVAD.cmk (a caspase-1 inhibitor)-treated rats (*P < 0.05). Compared with sham rats, LVPD-preload relationships in z-VAD.fmk (a broad-spectrum caspase inhibitor) + endotoxin and z-DEVD.cmk (a caspase-3 inhibitor) + endotoxin-treated rat hearts were statistically not different. No effects of caspase inhibitors on LV function was observed in sham rats (data not shown). B: determination of caspase-3-like activity. DEVDase enzymatic activity corresponding to caspase-3-like activity was measured, 8 h after in vivo treatments with endotoxin alone or with caspase inhibitors (z-VAD.fmk, a broad-spectrum caspase inhibitor; or z-DEVD.cmk, a caspase-3 inhibitor; or Ac-YVAD.cmk, a caspase-1 inhibitor) as described in MATERIALS AND METHODS. Results are expressed as picomoles of substrate p-NA hydrolyzed per microgram of proteins per minute. Data (means ± SE) of a representative experiment (n = 6 in each group) was duplicated. Comparisons between means were made by one-way ANOVA; *P < 0.05 for the Dunnett post hoc test comparing variable group with control group (sham-treated animals). C: quantification of apoptosis by DNA fragmentation assay (oligonucleosomes). Hearts from rats (n = 9; except for endotoxin + Ac-YVAD.cmk, n = 6) were submitted to ELISA test (MATERIALS AND METHODS) 8 h after treatment. Results are expressed as optical density (OD)/µg proteins. Results represent the means ± SE. Comparisons between means were made by one-way ANOVA; *P < 0.05 for the Dunnett post hoc test comparing variable group with control group (sham-treated animals).
CASPASES AND MYOCARDIAL DYSFUNCTION IN SEPSIS

Toxin-induced myocardial dysfunction, a role of caspase-1 is questionable based on the observation showing that Ac-YVAD.cmkk did not prevent myocardial dysfunction and apoptosis. These data are consistent with a previous report (10) indicating that Ac-YVAD.cmkk failed to protect mice from a lethal dose of endotoxin. In contrast, broad-spectrum z-VAD.fmkk and z-DEVD.cmkk inhibitors improved endotoxin-induced myocardial dysfunction. These caspase inhibitors were administered immediately, 2, and 4 h after endotoxin infusion. In this model, z-VAD.fmkk and z-DEVD.cmkk administered immediately and 2 h after endotoxin infusion prevented myocardial dysfunction and reduced significantly effector caspase-3 activation and nuclear apoptosis. When administered 4 h after endotoxin infusion, neither z-DEVD.cmkk nor z-VAD.fmkk reversed ongoing endotoxin-induced myocardial dysfunction and apoptosis. This observation suggests that after nuclear apoptosis apparition and caspase elevation caspase inhibition could no longer interfere to protect the heart from endotoxin. Thanks to the pattern of endotoxin-induced myocardial dysfunction, posttreatment with inhibitors of caspase could be only administered when myocardial dysfunction develops (immediately and 2 h after endotoxin infusion) but not in the setting of proven myocardial dysfunction (at 4 h post-endotoxin infusion).

In conclusion, these observations provide evidence that, in our model, caspase activation plays a role in the pathophysiology of endotoxin-induced myocardial dysfunction. The precise mechanisms of caspase-induced myocardial dysfunction and apoptosis warrant further investigation. However, caspase inhibition strategy may represent a novel therapeutic approach of endotoxin-induced myocardial dysfunction.

Fig. 4. Effects of injection of caspase inhibitors after endotoxin challenge on heart alterations. For each individual caspase inhibitors, a one-way ANOVA model was built with the time of injection of the caspase inhibitor being the grouping variable. Post hoc analyses were made using the Dunnett test comparing variable group with control group (sham-treated animals). Statistical significance was assigned to P < 0.05. A: 8 h after endotoxin (endo) challenge baseline measurements of changes in LV function (LVDP) were performed in hearts from rats treated with caspase inhibitors immediately (coinjection), 2, or 4 h after endotoxin treatment as explained in MATERIALS AND METHODS. Results obtained in hearts from rats treated with endotoxin (8 h) and caspase inhibitors were compared with those obtained after treatment with endotoxin alone (n = 6, mean LVDP value of 46 ± 5 mmHg) and expressed as the percentage of inhibition of this response [% of inhibition X = Y−Y(Z−Y), where X and Y correspond, respectively, to the observed mean LVDP value in hearts from rats treated with endotoxin alone (Y) and with caspase inhibitors immediately (coinjection), 2, or 4 h after endotoxin treatment (X)]. Results were corrected for the value of sham-treated rats (Z) [n = 6, mean LVDP value of 89 ± 5 mmHg]. *P < 0.05 compared with sham animals. B: 8 h after endotoxin challenge, DEVDase activity was measured in hearts from rats treated with caspase inhibitors in coinjection, 2, or 4 h after endotoxin treatment as explained in MATERIALS AND METHODS. Results obtained in hearts from rats treated with endotoxin (8 h) and caspase inhibitors were compared with those obtained after treatment with endotoxin alone (n = 6, mean DEVDase value of 52 ± 5 pmol·µg⁻¹·min⁻¹) and expressed as the percentage of inhibition of this response [% of inhibition X = Y−Y(Z−Y), where X and Y correspond, respectively, to the observed mean DEVDase value in hearts from rats treated with endotoxin alone (Y) and with caspase inhibitors immediately (coinjection), 2, or 4 h after endotoxin treatment (X)]. Results were corrected for the value of sham-treated rats (Z) [n = 6, mean DEVDase value of 15 ± 4 pmol·µg⁻¹·min⁻¹]. *P < 0.05 compared with sham animals. C: 8 h after endotoxin challenge, oligonucleosomal fragmentation was measured in hearts from rats treated with caspase inhibitors at coinjection, 2, or 4 h after endotoxin treatment as explained in MATERIALS AND METHODS. Results obtained in hearts from rats treated with endotoxin (8 h) and caspase inhibitors were compared with those obtained after treatment with endotoxin alone (n = 6, a mean value of 90 ± 8 OD/µg protein) and expressed as the percentage of inhibition of this response [% of inhibition X = Y−Y(Z−Y), where X and Y correspond, respectively, to the observed mean OD/µg protein value in hearts from rats treated with endotoxin alone (Y) and with caspase inhibitors immediately (coinjection), 2, or 4 h after endotoxin treatment (X)]. Results were corrected for the value of sham-treated rats (Z) [n = 6, mean value of 9 ± 2 OD/µg protein]. *P < 0.05 compared with sham animals.
REFERENCES


