Attenuation of Ischemia/Reperfusion Injury by a Caspase Inhibitor

To the Editor:

Yaoita et al\(^1\) present an interesting study of the attenuation of ischemia/reperfusion injury in rats by Z-Val-Ala-Asp (OMe)-CH\(_2\)F (ZVAD-fmk), a caspase inhibitor. In a previous study, Gottlieb et al\(^2\) reported that ZVAD-fmk was an apoptosis inhibitor in a preconditioning rabbit cardiomyocyte model.

Because ZVAD-fmk seemed to exhibit interesting properties in the inhibition of the caspase cascade, we undertook a study to inhibit apoptosis in an animal model.

ZVAD-fmk was directly purchased from the manufacturer (Enzyme Systems Products), and our first step was to check the purity of the compounds. High-performance liquid chromatography was performed on a \(\mu\)C18 column (Hypersil, England) with a mobile phase (ammonium acetate pH 4.4, 0.1 mol \(\cdot\) L\(^{-1}\)/acetonitrile: 40/60) pumping at 0.5 mL/min. Detection was performed with a photodiode array detector (Waters, France) for a 2-hour run. ZVAD-fmk was dissolved in dimethylsulfoxide and diluted with water at 2 mmol/L as recommended by the manufacturer. Three different peaks could be identified during the first 5 minutes, and no other peak in the remaining time. Since the manufacturer claimed a 91% purity, we repeated the same experiment with a different lot. Unfortunately, we obtained similar results with a purity <50%. According to the chromatograms sent by the manufacturer from their quality-control record, it seems that their thin-layer chromatography does not have sufficient resolution and therefore is not capable of checking the purity of the compounds.

ZVAD-fmk probably has the interesting biological properties that the 2 cited studies\(^1,2\) have reported, but the exact identification of the involved compounds remains to be performed. We would advise checking the purity of the compounds as a first step when ZVAD-fmk is used.

Weinmann et al\(^1\) pointed out that their lots of Z-Val-Ala-Asp(OMe)-CH\(_2\)F (ZVAD-fluoro-methylketone; ZVAD-fmk) were relatively impure. Our ZVAD-fmk was purchased from the same manufacturer, and we did not check the purity of the compound before use. ZVAD-fmk has been reported to inhibit DNA fragmentation in a variety of cell species at concentrations up to 100 \(\mu\)mol/L.\(^1,2\) In our previous report,\(^3\) the study was designed to achieve a relatively low circulating concentration of the compound (~20 \(\mu\)mol/L) because it was quite expensive. If the purity of the compound used by us was low, as they suggested, our results may have been obtained with a quite low concentration of the compound.

To confirm the effect of ZVAD-fmk, a dose-response relationship is required, and we determined this (unpublished) using a rat model consisting of a 30-minute coronary occlusion and a 24-hour reperfusion. We used the compound at a dose that was 2-fold higher than in our previous report.\(^3\) Infarct size/risk area on the triphenyl tetrazolium chloride and Evans blue staining and terminal deoxynucleotidyl transferase–mediated dUTP-biotin nick end labeling (TUNEL) positivity of cardiomyocytes within the risk area were 43.9 ± 4.7% and 2.5 ± 1.1%, respectively, with a dose of 6.6 mg/kg (n = 4); 52.4 ± 4.0% and 3.1 ± 0.9% with a dose of 3.3 mg/kg (not significant versus 6.6 mg/kg), and 66.6 ± 3.7% and 11.1 ± 1.0% with an inert vehicle (P < 0.01 versus 6.6 mg/kg, respectively) (n = 8 each). The effect of 6.6 mg/kg ZVAD-fmk was still modest in contrast to the greatest infarct-attenuating effect obtained by preconditioning (5 minutes’ ischemia and 10 minutes’ reperfusion), then 30 minutes’ ischemia followed by 24-hour reperfusion; infarct size/risk area 15.5 ± 0.02%, P < 0.01 versus 6.6 and 3.3 mg/kg ZVAD-fmk or vehicle; TUNEL positivity 1.5 ± 0.3%, P < 0.01 versus vehicle). Although differences between 6.6 and 3.3 mg/kg were not significant in this small experiment, there was a trend of dose-related infarct-size attenuation.

We appreciate the importance of the suggestion made by Weinmann et al. Depending on the purity of each lot, modification of the dose utilized must be taken into account for the precise assessment of the study.

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Circulation. 2000;101:e75
doi: 10.1161/01.CIR.101.5.e75
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
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