The inhibition of cell death lies at the heart of the interesting publication in this issue of Circulation by Huang et al.\textsuperscript{1} The authors’ major message is that Bcl-xL gene transfer prolongs the cold preservation time of rat hearts destined for transplantation. Bcl-xL belongs to the large Bcl-2 family and has been reported to inhibit Bax translocation to the mitochondria and to reduce cytochrome \(c\) release, thereby interrupting the apoptotic cascade and reducing the number of cells dying by apoptosis.\textsuperscript{2,3} This is precisely what Huang et al\textsuperscript{1} have shown—namely, that inhibition of the apoptotic pathway can partially prevent the deleterious effects of long-term ischemia by reducing the rate of apoptosis. In addition, these authors show that “infarct size” was reduced in treated hearts and that the rate of polymorphonuclear cell infiltration was minimal as compared with hearts without gene transfer. Infarct size was determined in Langendorff buffer-perfused hearts without any coronary artery ligation and describes the tissue area with necrotic cells as demonstrated by triphenyltetrazolium (TTC) staining. To the uninitiated reader it appears, therefore, that an intervention aimed at reducing apoptosis is also able to interfere with the process of ischemic cell death, ie, with oncosis (this is the preferred, modern term for necrosis; necrosis proper is the process of cell demise after any type of cell death\textsuperscript{4}). The number of cardiomyocytes dying by either cell death mechanism, apoptotic or onotic, was reduced: Only 6.5% of all cardiomyocytes were terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) positive in hearts with Bcl-xL gene transfer, as compared with 18.9% in the untreated hearts, and infarct size was 23% in the treated group versus 47.7% in the hearts without gene transfer. Creatine kinase (CK) activity measured in the coronary outflow of Langendorff-perfused hearts was likewise reduced in hearts with Bcl-xL gene transfer.

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The cell death paradox is here represented by the astounding phenomenon that apparently 2 modes of cell death, apoptotic and onotic, were influenced by Bcl-xL gene transfer, which should exclusively interfere with the apoptotic pathway. This is surprising given the well-known fact that both modes of cell death employ different mechanisms of initiation and cellular execution.\textsuperscript{5} Apoptosis is a programmed (suicidal), mostly caspase-driven and energy-dependent process, whereas onotic cell death is accidental (because ischemic injury is not preprogrammed), is independent of caspase activation, and occurs subsequent to ATP depletion.\textsuperscript{6} In addition, a third type of cellular demise has been described, autophagic cell death, but this may not be of great importance in the present animal model because it occurs mostly in tissue exhibiting chronic degeneration, such as Alzheimer’s disease in the brain and failure of the human heart.\textsuperscript{7,8}

Huang et al\textsuperscript{1} showed that Bcl-xL gene transfer prevented Bax loss from the cytosol and decreased cytochrome \(c\) release from the mitochondria, whereas Bax was translocated from the cytosol to the mitochondria and caused massive cytochrome \(c\) release in untreated hearts.\textsuperscript{1} These data are in line with accumulating evidence suggesting an intimate interrelationship of apoptosis and mitochondrial function that occurs at the interface between Bcl-2 family proteins and the outer mitochondrial membrane protein, namely voltage-dependent anion channel (VDAC). Although Bcl-xL was shown to stimulate VDAC closure, thereby preventing mitochondrial alterations in response to death stimuli, Bax protein promotes its opening to release cytochrome \(c\) and promote apoptosis.\textsuperscript{9}

In the accompanying article by Huang et al,\textsuperscript{1} however, there is no clear-cut explanation of the simultaneous occurrence of apoptosis and oncosis and their inhibition by Bcl-xL gene transfer. Because, unfortunately, myocardial ATP levels were not measured, the explanation of these puzzling results must be speculative. Referring to the work of Tatsumi et al\textsuperscript{10} and Kuznetsov et al,\textsuperscript{11} Huang et al believe that the inhibition of cytochrome \(c\) release and preservation of mitochondrial function are decisive in preventing myocardial injury. Kuznetsov et al reported that cytochrome \(c\) release from the mitochondria, usually held to be a major proapoptotic event, caused necrosis by reducing ATP levels, and that the heterogeneity and extent of cytochrome \(c\) release regulate the switch between apoptosis and necrosis.\textsuperscript{11} Tatsumi et al\textsuperscript{10} reported that intracellular ATP plays an important role in the execution of apoptosis and not of necrosis (or oncosis), confirming earlier results by Leist et al.\textsuperscript{12} Tatsumi and colleagues demonstrated in neonatal cardiomyocytes a distinct correlation between the rates of occurrence of apoptotic cell death and ATP levels (ie, ATP is necessary for apoptosis to take place and more apoptosis occurs at higher ATP levels). Furthermore, they showed that necrosis appeared exclusively in the presence of total glucose deprivation accompanied by total loss of ATP. They also demonstrated that constitutively expressed caspase-3 is not activated in the

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absence of ATP; however, cleavage of the procaspase (32 kDa) resulting in the activated form of caspase-3 (17 kDa) was observed in the presence of higher ATP levels that resulted in apoptotic cell death. Thus, it appears that oncosis occurs at low to zero ATP levels and apoptosis can only occur in the presence of higher intracellular energy stores.

When transferring this knowledge to the data presented by Huang et al., it must be concluded that ATP levels in both treated and untreated hearts must have been different in different areas of the myocardium, resulting in either necrotic or apoptotic cell death. Their results furthermore suggest that ATP levels in the myocardium treated with Bcl-xL gene transfer were elevated because infarct size (ie, the area of necrotic cells) was reduced in this situation. Because the number of TUNEL-positive cells was lower in hearts with Bcl-xL gene transfer, ATP levels should have been high enough to preserve the tissue and to prevent the occurrence of apoptosis. Because ATP values are unknown and caspase-3 and its degree of activation were not determined, however, this attempt to explain the data presented by Huang et al remains purely speculative and more work is needed to clarify this issue.

Furthermore, in the work of Tatsumi et al there is no evidence-based reason that allows the conclusion that necrosis and apoptosis share common initial mechanisms. In our opinion, this proposal is not very likely because one of the critical differences between these 2 modes of cell death is that damage to the sarcolemma resulting in cell membrane leakiness is one of the major and early hallmarks of oncocytic cell death, whereas in apoptosis the membrane remains intact and functional. The authors noticed neither a simultaneous occurrence of apoptosis and necrosis in the same culture dish nor the transition of necrosis to apoptosis or vice versa in the same cell. For this reason it must be concluded from their work that cells die either by apoptosis or by necrosis, according to the intracellular energy level. A common mechanism of initiation is questionable.

There is ongoing discussion of whether apoptosis or necrosis is the primary form of cell death after myocardial injury, especially in the setting of ischemia/reperfusion. This may be the result of varying experimental design as well as unsolved technical issues in determining the mode and rate of cell death.

The problem of the no-reflow phenomenon is inherent to models of long-term ischemia followed by reperfusion. Ischemia causes injury not only to the cardiomyocytes but also to capillaries and the arteriolar endothelium so that microvascular edema is common in ischemic myocardium. This cell swelling obliterates the vascular lumen and prevents tissue perfusion during reperfusion. The TTC reaction used to show the occurrence of necrotic cells may then show false-positive results. In other words, dead tissue, instead of being TTC positive, shows the brick red positive stain because NADH (or NAD) has not been washed out. A reperfusion marker such as fluorescein should have been used to show that tissue perfusion is reestablished and uniform in the areas with positive TTC reaction. It cannot be excluded (but again, this is purely speculative) that endothelial edema was reduced by Bcl-xL gene transfer and that the TTC reaction to some extent gave false-positive results. The mechanism of action of Bcl-xL gene transfer on endothelial cells, however, remains unknown and its likelihood most probably is low.

Unsolved technical problems in determining the rate of apoptosis may also play a role. The TUNEL reaction has been shown to label not only apoptotic but also necrotic cells, as well as those undergoing DNA replication and this may result in false-positive numbers. For a positive control of this technique, the use of tissue with a known rate of apoptosis, such as small intestine, is strongly recommended. The possibility that TUNEL and TTC reaction may be potentially misleading should be kept in mind to exclude that the data presented may be subject to artifacts.

In conclusion, the work by Huang et al is not only interesting but also thought provoking because of the astonishing effect that antiapoptotic treatment is able to reduce the rate of apoptotic and oncocytic (ischemic) cell death. Mechanisms of action are unknown at present, and an explanation is hampered by the lack of data on local ATP content and the activation of caspase-3. The discussion of the role of apoptotic, necrotic, and autophagic cell death in ischemia/reperfusion is ongoing, and before any therapeutic principles are recommended or used in human studies, the apparent cell death paradox should be explored and clarified in more detail in future studies.

References


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Cell Death and Adenosine Triphosphate: The Paradox
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