Letters to the Editor

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Poly–adenosine diphosphate–ribose polymerase inhibition for myocardial protection: Pathophysiologic and physiologic considerations

To the Editor:

We read with great interest the article by Szabo and associates[1] in which they reported the use of state-of-the-art animal instrumentation and molecular biologic techniques to demonstrate that poly–adenosine diphosphate–ribose polymerase (PARP) inhibition protects against myocardial and endothelial reperfusion injury after crystalloid cardioplegic arrest. In the discussion, they attributed this primarily to energy-saving effects, because activation and (more importantly) activity of the enzyme PARP require energy.[1] However, they did not address the physiologic functions of PARP in repairing DNA breaks, especially after DNA cleavage by caspases as part of the apoptotic cascade, a genetically programmed process for the death and subsequent removal of injured cells.[2] Recent studies have shown that cardioplegic arrest induces the apoptosis signal cascade in cardiac myocytes and endothelial cells,[3,4] so inhibition of the repair enzyme PARP—even though energy sparing—may result in higher numbers of cardiac myocytes completing apoptosis, thus abolishing any potential positive short-term effects attributable to PARP inhibition.

Considering that the benefit of energy sparing by PARP inhibition is necrosis avoidance,[5] the therapeutic concept of PARP inhibition can only be effective if the unprotected ischemia/reperfusion injury leads to necrosis, such as in myocardial infarction or stroke. If, however, PARP activation does not lead to cellular oxidized nicotinamide adenine dinucleotide (NAD^+) depletion by more than 75%,[5] as has recently been demonstrated in transient global cerebral ischemia, PARP inhibition decreases the number of surviving cells.[6] These data suggest that in the absence of NAD^+ depletion as induced by mild ischemia-reperfusion injury (protected ischemia such as cardioplegic arrest) PARP activation would be protective. Therefore, the therapeutic use of PARP inhibitors should be critically proved with respect to the degree of injury. Finally, it must be questioned whether inhibition or scavenging of upstream effectors of apoptosis, such as reactive oxygen species, would be more effective through reduction of endogenous PARP activation parallel to the inhibition of DNA damage and subsequent apoptosis. A study comparing inhibition of upstream activators of ischemia-reperfusion injury (reactive oxygen species scavengers) versus PARP inhibition appears appropriate. Further issues that need to be addressed before therapeutic PARP inhibition are the various other physiologic functions of activated PARP, including transcriptional regulation, stimulation of nuclear proteasomal function, and its anti-aging effect.[2] Certainly, these aspects are beyond the scope of the study of Szabo and associates; however, they should have been mentioned.

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Reply to the Editor:

In response to the comments of Bloch and Mehlhorn on the effects of poly–adenosine diphosphate–ribose polymerase (PARP) inhibition after cardioplegic arrest and reperfusion, we agree that the PARP enzyme family has a complex regulatory role under several physiologic and pathologic conditions. After a decade of intensive research, we reported on the role of PARP in general[1] and focusing on ischemia-reperfusion injury.[2,3] To date, the role of PARP in physiologic DNA repair, apoptosis, and necrosis can be summarized as follows: depending on the severity of DNA damage, genotoxic stimuli can trigger three different pathways. In the case of mild DNA damage, PARP facilitates DNA repair and thus survival. However, the exact physiologic role of PARP still remains to be clarified; many authors have suggested that PARP is an abundant enzyme with limited role under physiologic conditions. More severe DNA damage induces apoptotic cell death, during which caspases, the main executor enzymes of apoptotic process, inactivate PARP, cleaving it into two fragments (p89 and p24) and thus PARP *cleavage* by caspases is a marker of *apoptotic* cell death. This pathway allows cells with irreparable DNA damage to become eliminated in a safe way. Much of the cell death related literature focuses on PARP *cleavage* (as opposed to PARP *activation*). The most severe DNA damage may cause excessive PARP activation, depleting oxidized nicotinamide adenine dinucleotide (NAD^+) and adenosine triphosphate stores. NAD^+/ATP depletion blocks apoptosis and results in necrosis. This pathway, which we proposed in the article under discussion, has no relationship to the PARP cleavage pathway. As mentioned previously, the cleaved form of PARP is catalytically inactive; PARP cleavage has been considered as an endogenous mechanism that serves to prevent PARP-dependent metabolic suppression and necrosis.

Considering the previously mentioned mechanisms, the cited study of Fischer and colleagues[4] is not contrary to but supportive of our results. They found caspase activation but *no* apoptotic cell death and *no* PARP *cleavage* in a similar model of cardiopulmonary bypass and cardiac arrest. On the basis of these findings, Bloch and Mehlhorn criticized our study, suggesting that pharmacologic PARP inhibition may be detrimental by inhibiting physiologic DNA repair and promoting apoptosis.

We do believe that the fact they did not find apoptotic cell death and PARP *cleavage* (inactivated PARP) after cardioplegic arrest indicates that the primary form of cell damage is the necrotic (or prenecrotic) pathway, with concomitant PARP *activation* instead of PARP *cleavage.* It is also evident that cardioplegic arrest leads to adenosine triphosphate depletion, and therefore it is not surprising that no or negligible apoptosis occurs during reperfusion, because apoptosis is an adenosine triphosphate–dependent process. The loss of the ability of the cells to undergo apoptosis results in missing PARP *cleavage* and turns the cells toward PARP *activation* and metabolic suppression. Indeed, there is immunohistochemical evidence of PARP *activation* in different models of cardioplegic arrest and reperfusion.[2,5] It is also important to note that cellular PARP activation does not always need to culminate in full-fledged cellular necrosis (complete breakdown of cell membrane integrity and release of cellular content into the extracellular space); a partial, and reversible suppression of cellular energetic pools can be associated with reversible cell dysfunction. These phenomena are sometimes termed *prenecrosis* or *cytopathic hypoxia* and are reversible by PARP inhibition.[6,7]

The questions raised about additional functions of PARP, its potential toxicity, and its role in aging were far beyond the scope of our study; however, we refer to a previous review article in which they were discussed and considered in detail.[1] We note, nevertheless, that in addition to participating in the NAD depletion/prenecrosis/necrosis pathway PARP *activity* plays an active role in the transcription of various proinflammatory genes. Suppression of this process thus may provide additional cardioprotective benefits during and after cardioplegia.

The reference made by Bloch and Mehlhorn to stroke and NAD depletion does not have any direct relationship to our current work, but for the record we note that the vast majority of studies (more than 20 published reports, reviewed in Virág and Szabó[1]) demonstrate the marked protective effect of PARP inhibitors and genetic PARP deficiency in various models of stroke. In most experimental models of stroke (similar to myocardial ischemia) a prenecrotic/necrotic pathway of cell death (as opposed to apoptosis or PARP cleavage) appears to play the dominant role and can be beneficially affected by PARP inhibition.

Whether inhibition or scavenging of upstream effectors of apoptosis or necrosis, such as reactive oxygen species, would be more effective by reduction of endogenous PARP activation in addition to the inhibition of DNA damage and subsequent cell death remains to be clarified. We have preclinical experience with the novel porphyrinic peroxynitrite decomposition catalyst FP15,[8,9] which shows beneficial effects during regional ischemia/reperfusion[8,9] and after cardioplegic arrest (unpublished observations).

In conclusion, we maintain that PARP inhibition is a promising concept for reducing cell damage during ischemia and reperfusion. Even if alternative cardiac surgical techniques such as beating-heart surgery minimize ischemia-reperfusion injury, PARP inhibition may improve postoperative outcome in those cases in which cardioplegia is still necessary.

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