Does Inhibition of Glycogen Synthase Kinase Protect in Mice?

Elizabeth Murphy, Charles Steenbergen

Preconditioning (PreC) and postconditioning (PostC) have been shown to initiate a number of signaling cascades that reduce cell death. However, the mechanisms by which these signals reduce cell death have been elusive. PreC has been shown to phosphorylate and thereby inhibit glycogen synthase kinase (GSK)-3β, and perfusion with GSK inhibitors has been shown to reduce cell death induced by ischemia/reperfusion, when added before ischemia or when added at the start of reperfusion. These studies are consistent with data in other tissues showing that inhibition of GSK-3β reduces apoptosis. Information regarding the mechanism by which inhibition of GSK protects has been provided by Juhaszova et al, who report that inhibition of GSK-3β delays the opening of the mitochondrial permeability transition pore (MPT) (see the Figure). The MPT is a large-conductance pore in the inner mitochondrial membrane which is opened under conditions associated with ischemia/reperfusion, such as high matrix reactive oxygen species and high matrix calcium. Pharmacological inhibitors of the MPT have been shown to reduce ischemia/reperfusion injury, suggesting that activation of MPT might have a role in ischemia/reperfusion-mediated cell death. However the molecular components of the MPT have not been identified.

Juhaszova et al showed that myocytes isolated from mice with cardiac specific overexpression of a constitutively active form of GSK-3β, in which the serine 9 is replaced with alanine, are not protected by PreC or diazoxide. Juhaszova et al also decreased GSK-3β using interfering RNA and showed that this was protective, whereas decreasing GSK-3α was without effect. These data agree with data from other groups showing that inhibitors of GSK protect and that many types of cardioprotection result in increased phosphorylation of GSK-3β. However, the obligatory role of phosphorylation and/or inhibition of GSK in cardioprotection has been questioned by Nishino et al in this issue of Circulation Research. Nishino et al used GSK-3α/β knock-in (KI) mice in which the phosphorylation sites on GSK-3α (Ser21) and GSK-3β (Ser9) are changed to alanine. For controls, they used WT mice that were inbred from the same colony but were not littermates. In the GSK double KI mice, infarct size, measured in a Langendorff model of global ischemia and reperfusion, was significantly lower in PreC (21.9%) and PostC (22.2%) hearts compared to nonconditioned hearts (39.5%), calling into question whether phosphorylation or inhibition of GSK is required for protection in mice. The authors further tested the involvement of GSK inhibition in cardioprotection using pharmacological GSK inhibitors and found that GSK inhibitors were not protective in this species, even though they observed protection in rats. Thus, these data suggest a species difference in the role of GSK in ischemia/reperfusion injury.

In contrast to the study by Nishino et al, others have found that GSK inhibition is critical for cardioprotection in mice. In addition to the study by Sollott and colleagues, a recent study by Gomez et al found that inhibitors of GSK reduced infarct size in mouse heart.
The role of GSK in cardioprotection has been addressed in two ways. One approach is through genetic manipulation and the other approach is to use pharmacological agents. Each approach has advantages and disadvantages.

There are 2 genetic models that have been used to study the role of GSK in myocardial ischemia/reperfusion injury. Two groups (Juhaszova et al and Gomez et al) have used transgenic mice with cardiac-specific expression of a constitutively active form of GSK-3β, which cannot be phosphorylated. These studies find that many types of cardioprotection such as PreC and PostC do not confer protection in hearts and cardiomyocytes with constitutively active GSK-3β. Nishino et al used a different model: a genetically modified mouse with a knock-in of a GSK-3α and GSK-3β that cannot be phosphorylated. With this mouse model, PreC and PostC are not blocked; both reduce infarct size. Nishino et al have suggested that in the mouse model used by Juhaszova et al and Gomez et al that the overexpression of constitutively active GSK-3β results in an increase in atrial natriuretic factor and other factors that might alter cardioprotective signaling independent of GSK-3β. Indeed, Gomez et al found a decrease in fractional shortening in the mice overexpressing active GSK-3β. However, one might also question whether protective compensatory pathways are activated in the double KI mice used by Nishino based on the reduced infarct size observed in the double KI (39.5%) compared to wild-type (WT) (61.1%) hearts from the same colony. The finding of protection in the KI mice in the absence of PreC or PostC might suggest that protective pathways are activated in these mice, although PreC and PostC can enhance this protection and the enhanced protection by PreC and PostC is not lost in the KI mice. It is possible that the double KI mice have activation of protective signaling pathways and that these protective pathways intervene at a point downstream or parallel from GSK (see the Figure). Thus, loss of GSK inhibition would not inhibit protection if the signaling pathways downstream or independent of GSK were endogenously activated in the KI mice. It is unfortunate that the complex breeding strategy did not allow direct comparison with WT littermates. It should also be noted that Gomez et al did not use littermates. Thus, we are left with 2 different genetic models that give different results. Unfortunately, both genetic models are likely to have compensatory changes that can influence the interpretation. When genetically modified mice were first introduced, they were hailed as a way to test the effects of inhibiting pathways without the nonspecific side effects that can occur with inhibitors. However, as we have learned over the years, genetic modification, particularly those that are present through the life of the animal, result in many compensatory changes that make it difficult to draw unambiguous conclusions. So, unfortunately, the question of whether GSK inactivation is required for cardioprotection in the mouse or other species is not resolved and will require further study. In addition to the possibility that compensatory changes are being induced, there is also the concern that true protective in transgenic mice with overexpression of the same noninhibitable GSK-3β as used by Sollott and colleagues. Thus, there are conflicting data on the role of GSK-3β in ischemia/reperfusion injury in the mouse heart.

So what can we conclude regarding the role of GSK in cardioprotection? It is not unusual for there to be conflicting results concerning the role of kinases in cardioprotection and reduction in apoptosis. Until we better understand the details of the mechanisms by which myocytes die, it will be difficult to conclusively prove a requirement for any kinase. The majority of studies seem to suggest a role for GSK in cardioprotection. The lack of protection in the study by Nishino et al might reflect compensatory protection in the GSK KI mice that might have protected at a point downstream or independent of GSK. The lack of protection by inhibitors might reflect a lack of optimization of the dosing, although the authors did show that these concentrations of GSK inhibitors blocked phosphorylation of glycogen synthase. As illustrated in the Figure, it is also possible that GSK inhibition can initiate protection, but it is not unique, and there are other signaling pathways that can mediate protection. If these other pathways are upregulated in the KI mice, it might be that under these conditions, GSK inhibition is not required. It is also possible that the exact mix of protective kinases that are required for protection is model and species dependent. In other words, there may be multiple mecha-
nisms that can result in inhibition of the MPT. Under some conditions, inhibition of GSK is a major and essential part of this protection; however, under other conditions other pathways may become more important and thus GSK is not required.

In summary, the study by Nishino et al\textsuperscript{8} questions whether GSK inhibition is required for protection in mouse hearts. The lack of protection by GSK inhibitors observed by Nishino et al\textsuperscript{8} contrasts with that observed in other species (in fact Nishino et al found GSK inhibitors protect in rat) and with the protection reported by Gomez et al\textsuperscript{5} in mouse. Additional studies will be needed to resolve the role of GSK inhibition in mouse. Interestingly, the use of genetically modified mice to address the role of GSK in cardioprotection has not resolved this issue because of the compensatory mechanisms that appear to be present in the genetically modified mice. So, how do we resolve the role of GSK, or any kinase in protection? Clearly, a combination of genetically modified mice and pharmacological inhibitors is the present state-of-the-art. Additional studies, possibly using conditional KI of nonphosphorylatable GSK or adenoviral transfection of hearts or myocytes, along with pharmacological inhibitors, will be needed to resolve this issue.

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References

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