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The Role of Co-morbidities in Cardioprotection

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Abstract

Cardioprotective strategies such as pre and postconditioning result in a robust reduction in infarct size in young, healthy male animals. However there are data suggesting that the protection is diminished in animals with co-morbidities such as hypertension, hypercholesterolemia and diabetes. It is important to understand at a mechanistic level the reasons for these differences. The effects of sex and diseases need to be considered in design of cardioprotective interventions in animal studies and clinical trials.

Introduction

A number of agents have been shown to reduce infarct size in animal models but have failed to demonstrate protection in human clinical trials¹. A number of reasons for this discrepancy have been discussed. One important factor is the timing of administration of the drug^{2, 3}. Many drugs have been shown to be protective when given before reperfusion or immediately on the start of reperfusion, but animal studies have shown that the protection of most, if not all, cardioprotective agents is lost if they are given more than 10 minutes after the start of reperfusion. However, in many of the failed clinical trials these drugs were administered outside of the time window that had been shown to be protective in the animal studies.

Another reason for the discrepant results is that the animal studies were performed on young healthy animals whereas the humans who are treated with cardioprotective drugs tend to be older and have a number of co-morbidities^{1, 4-6}. The focus of this review will be to discuss the role of these co-morbidities in modulating cardioprotection. The confounding factors to be discussed include age, sex, and co-morbidities of diabetes, hypercholesterolemia and obesity.

The role of Sex in Cardioprotection

Females have been shown to exhibit endogenous cardioprotection; in many studies females have been shown to have smaller infarcts than males⁷⁻⁹. Addition of estrogen has also been shown to reduce infarct size^{10, 11}. Because of this endogenous protection in females, preconditioning and other cardioprotective approaches have a smaller protective effect. However, most studies find that preconditioning reduces infarct size in both males and females¹²⁻¹⁴. For example, as illustrated by the data of Taldukder et al¹² although infarct size is smaller in females than males (~32% in females versus ~42% in males), infarct size is reduced by preconditioning in both males (~14%) and females (17%). Penna et al found similar results for postconditioning¹³, and Shinmura et al found similar effects for opioid induced late preconditioning¹⁴. The reduced protection by preconditioning and postconditioning in females is likely due to overlap between cardioprotective signaling in females and pre- and postconditioning signaling. If cardioprotective signaling pathways are endogenously activated in females, then pre- and postconditioning will result in a smaller

percentage reduction in infarct size. Thus a loss of cardioprotection can be due to activation of endogenous protection (as in females) in which case the protection is reduced because of an increase in baseline protection, so in some conditions it is more difficult to detect a significant protection. Indeed, the signaling pathways that have been shown to result in cardioprotection with pre and post-conditioning and many pharmacological preconditioning agents are the same pathways that are upregulated in females and suggested to be involved in the protection in females. For example, nitric oxide has been shown to elicit cardioprotection¹⁵. It has been reported that eNOS is required for acute in vivo preconditioning of the heart¹². Furthermore, females have been reported to have elevated levels of eNOS⁹. The increased eNOS in females at baseline contributes to endogenous protection in females, but since pre- and postconditioning also protect via NOS activation there will be less protection (as a percentage of baseline protection) with pre- and postconditioning. As protection in females is lost in ovariectomized (OVX) animals, one would expect that PC would be more protective in OVX females than in non-OVX females. However, this was not found to be the case due to reduced activation of PKC signaling in OVX females¹⁶. It is worth noting that cardiovascular disease typically occurs in post-menopausal females, and therefore the cardioprotective effects of estrogen observed in premenopausal women is lost. It is of interest to consider that some of the same co-morbidities that interfere with the cardioprotection in aged males may also be responsible for the lack of protection observed by hormone replacement therapy in post-menopausal women.

The Role of Hypercholesterolemia

Hypercholesterolemia is a commonly found in patients with cardiovascular disease and is considered to be a risk factor for cardiovascular disease¹⁷. Indeed the statin drugs work at least in part by reduced serum cholesterol. A number of studies have reported that preconditioning and/or postconditioning do not reduce infarct size in animals with hypercholesterolemia^{18–24}. Iliodromitis et al reported that hypercholesterolemia blocked the protection afforded by postconditioning, but not by preconditioning¹⁹. Kupai et al also reported that postconditioning was blunted with hypercholesterolemia²³. Tang et al reported that hypercholesterolemia impaired late preconditioning by a tetrahydrobiopterin (BH4) dependent mechanism¹⁸. Pacing induced preconditioning was not protective in atherosclerotic rabbits and this loss of protection was attributed to hypercholesterolemia²⁰. Preconditioning in patients by coronary angioplasty was also found to be reduced by the presence of hypercholesterolemia²¹. In contrast to these studies suggesting that hypercholesterolemia reduces the protection, Donato et al report that a high cholesterol diet does not block postconditioning²⁵.

Thus co-morbidities such as hypercholesterolemia, reduce cardioprotection^{18–24}, but in contrast to the reduced protection in females, hypercholesterolemia does not provide endogenous protection instead it appears to block the cardioprotective signaling and thereby block the protection. With hypercholesterolemia the infarct size in non-preconditioned hearts is similar, but there is no infarct size reduction with preconditioning. It is important to understand the mechanism by which co-morbidities such as hypercholesterolemia block cardioprotection. For example if as suggested by Tang et al the lack of protection is due to loss or oxidation of BH4 (see figure 1), which will block nitric oxide signaling and increase ROS productin, we can intervene by administration of BH4 to restore protection in diseased animals.

Diabetes and Obesity

The prevalence of obesity and associated type 2 diabetes mellitus are increasing at a prodigious rate worldwide. These cardiovascular risk factors are associated with worse outcomes in patients that develop coronary ischemia and even increase the risk of complications from interventions to restore coronary flow such as angioplasty and coronary artery bypass grafting^{26,27}. Obesity and type 2 diabetes mellitus predispose to a worse outcome from ischemia and reperfusion injury in part due to their direct predisposition to other cardiac risk factors such as hypercholesterolemia, hypertension and hypercoagulability. Additionally, obesity and diabetes appear to perturb the functioning of mitochondria via numerous mechanisms^{28,29}. This intracellular organelle, in turn modulates susceptibility to tissue injury due to ischemia-reperfusion as the mitochondria balance cellular energetics, responses to reactive oxygen species and cellular repair programs such as mitophagy and cell death programs including apoptosis^{30–32}. Furthermore, diabetes and obesity have also been shown to lead to a decrease in BH4 and uncoupling of NOS^{33–36}. Mitochondrial dysfunction can lead to increase ROS leading to oxidation and loss of BH4 leading to uncoupling of NOS which reduces nitric oxide generation and leads to increased ROS generation by NOS (see Figure 1B).

Interestingly, mitochondrial biology per se is important in modulating adaptability to the cardioprotective programs of ischemic preconditioning and postconditioning. Hence, it would not be surprising if the conditions of obesity and diabetes attenuate the capacity for these preconditioning triggers to confer myocardial protection in animals and human subjects with obesity and type 2 diabetes mellitus^{37–39}. Although the mechanism may not be completely synonymous animals with type I diabetes similarly shows resilience to protection from ischemic preconditioning³⁹.

Aging as a risk factor attenuating preconditioning like cardioprotection

Many factors will play a role in attenuating preconditioning-induced cardioprotection in the elderly and these include the risk factors described above, including hypercholesterolemia and increased prevalence of type 2 diabetes mellitus. Interestingly, mitochondrial homeostasis and plasticity are also impaired in the elderly and this is thought to not only play a role in the overall decline in cellular function but may additionally add a self-perpetuating effect where mitochondrial dysfunction promotes increased reactive oxygen species levels, which in turn progressively disrupt mitochondrial function^{40,41}. Tetrahydrobiopterin has also been shown to be reduced with aging leading to uncoupling of NOS resulting in decreased nitric oxide production and increased ROS production^{33,42,43}. The lack of nitric oxide production by NOS could interfere with cardioprotective signaling, and the increase in ROS could exacerbate the injury.

In this light, in animal models of aging, even without the concomitant risk factors such as hypercholesterolemia and diabetes, we find that the capacity to evoke preconditioning like protection is diminished⁴⁴ and strategies that promote the restoration of mitochondrial functioning such as caloric restriction and resveratrol does appear to have ameliorative effects^{45–47}. Also administration of BH2 or nitrite has also been shown to restore vascular responsiveness.³³

Conclusion

The sex-difference in receptiveness towards preconditioning-induced cardioprotection is a unique factor in that the female gender confers innate protection that ameliorates the added benefit of cardioprotective strategies. Cognizance of this gender-specific benefit of the female sex is important in designing studies in animals and human's to increase the number

of female subjects to establish clear therapeutic benefit. The other ‘co-morbidities’ discussed in this review, i.e. hypercholesterolemia, obesity, diabetes and aging all increase resistance to the induction of cardioprotection from preconditioning like triggers. As these co-morbidities are very prevalent in human populations at risk for cardiovascular disease, the use of animal models with these comorbidities may give rise to more robust preclinical data to then extend to clinical trials. Additionally, as the pathophysiology underpinning of how these comorbidities perturb adaptability to preconditioning like triggers are delineated, these pathways or organelles e.g. mitochondria may identify novel targets to modulate to enhance ischemia-reperfusion resilience.

As shown in figure 1, pre- and postconditioning both activate signaling pathways that lead to an increase in NOS. Hypercholesterolemia, diabetes, obesity hypertension and aging are all associated with mitochondrial dysfunction and loss of the NOS cofactor, BH4. The loss of BH4, this could explain the lack of protection by pre and postconditioning with aging, hypertension, hypercholesterolemia, obesity and diabetes^{18, 33–35, 43, 48–51}. Loss of BH4 uncouples NOS such that it produces less NO and increasing amounts of ROS⁵². Thus signaling pathways that activate NOS no longer generate cardioprotective levels of NO, but instead generate more ROS. BH4 administration has been shown to re-couples NOS and ameliorate hypertrophy due to pressure overload⁵³. Interestingly, BH4 levels are also depleted in post-menopausal women and this has been suggested to contribute to the lack of protection of estrogen given in hormone replacement therapy to this group of women⁵⁴. BH4 co-administration with cardioprotective drugs may enhance these cardioprotective strategies in patients with common co-morbidities.

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Figure.

Figure 1a shows that activation of NOS is a common mechanism in most cardioprotective signaling. Activation of NOS results in generation of low levels of nitric oxide that leads to cardioprotection. With co-morbidities (Figure 2b) such as aging, hypercholesterolemia, hypertension, diabetes, and obesity there is mitochondrial dysfunction or other mechanisms resulting in increased oxidation of tetrahydrobiopterin (BH₄) leading to reduced BH₄ levels in the cell. The loss of BH₄, a cofactors for NOS, leads to uncoupled NOS activity in which the enzyme now produced ROS rather than NO. This loss of NO signaling could explain the lack of protection with cardioprotective signaling in the presence of these co-morbidities.