

## CLINICAL APPLICATION OF ISCHEMIC PRECONDITIONING IN THE ELDERLY

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- A mild stress such as brief ischemic episodes may protect the heart from a successive and more prolonged myocardial ischemia (ischemic preconditioning). This phenomenon is considered a typical “hormetic mechanism” by which the heart is immunized from pathological insults such as myocardial ischemia. This mechanism is reduced with aging and it may be restored and/or preserved by drugs such as adenosine or nicorandil, a mitochondrial  $K_{ATP}$  channels, and lifestyle interventions such as physical activity and/or hypocaloric diet. Moreover, since the mechanisms involved in cardiac ischemic preconditioning have been established basic and clinical investigators are encouraged to test several drug in well-controlled animal and human studies in order to prevent and/or restore the age-related reduction of ischemic preconditioning.

*Key words: hormesis, preconditioning, aging, lifestyles*

## **INTRODUCTION**

Ischemic preconditioning (IP) is an adaptive mechanism in response to brief episodes of myocardial ischemia able to reduce the cellular damage subsequent to a more prolonged ischemic damage firstly described by Murry *et al.* (1986). Thus, a brief period of ischemia and the following reperfusion makes the heart more resistant to successive more prolonged ischemic insult and it reduces the infarct size (Napoli *et al.* 2000; Lakatta and Sollott, 2002; Yellon and Downey, 2003). IP represents a classical example of hormetic effect of a mild stress (i.e. brief and multiple ischemic episodes) able to get a protection in the heart against the more prolonged ischemic insult.

This mechanism does not depend on collateral vessels since it is present in animal models without collateral vessel development and in experimental model as in the isolated perfused heart subjected to a global ischemia (Napoli *et al.* 2000; Lakatta and Sollott, 2002; Yellon and Downey, 2003). The protective effect of ischemic preconditioning could be reduced if the time between preconditioning ischemic episode and the prolonged ischemic episode is excessive. Finally, ischemic preconditioning is classified in “early” when the protective effect is manifest immediately from ischemic episode and “delayed” when the protective effect is manifest 24 hours from ischemic episodes (Napoli *et al.* 2000; Lakatta and Sollott, 2002; Yellon and Downey, 2003).

## **IP CLINICAL EQUIVALENTS**

Preinfarction angina, warm-up phenomenon, walk-through angina and transluminal coronary angioplasty are considered clinical equivalents of IP (Napoli *et al.* 2000; Yellon and Downey, 2003). The incidence of mortality and cardiogenic shock is reduced in patients with preinfarction angina (Kloner *et al.* 1995) and patients with preinfarction angina treated with thrombolytic therapy showed a more rapid reperfusion and a reduction of infarct size (Ottani *et al.* 1995; Andreotti *et al.* 1996). IP is clinically involved also in “walk-through angina”, i.e. an effort angina fol-

lowing physical exercise which paradoxically disappears when the exercise keeps on going, in “warm-up phenomenon”, i.e. a reduction of clinical and electrocardiographic modifications of effort ischemia following the first exercise test, and “transluminal coronary angioplasty”, i.e. a reduction of electrocardiographic, biochemical and clinical signs of ischemia after the first balloon inflation (Napoli *et al.* 2000; Lakatta and Sollott, 2002; Yellon and Downey, 2003).

## **IP MECHANISM**

IP is mediated by several agonists (adenosine, norepinephrine, bradykinin, opioids, ect.) which activate G-protein-coupled receptors (GPCR) that in turns activate phosphoinositide-3-kinase (PI3K)/serine/threonine kinase (Akt) with subsequent activation of nitric oxide (NO) synthase and NO formation, guanylate cyclase, protein kinase G (PKG) and Protein Kinase C (PKC). Moreover, PKC should be also directly activated through adenosine or guanylate cyclase by natriuretic peptide receptor. PKC shifts to  $\alpha$ -PKC leading to the opening of the mitochondrial ATP-dependent potassium channels (mitoK<sub>ATP</sub>) that is responsible to somehow lead to the production of reactive oxygen species (ROS) (Juhaszova *et al.* 2005; Downey *et al.* 2007; Heusch *et al.* 2008). ROS activate p38 mitogen-activated kinase and “priming” of mitochondrial permeability transition pore (MPTP) is initiated (Juhaszova *et al.* 2005; Downey *et al.* 2007; Heusch *et al.* 2008). The MPTPs are multiprotein complexes forming non-selective pores in the inner membrane of the mitochondria that allows free passage of any molecule which disrupts the permeability barrier of the inner membrane with devastating consequences related to the uncoupling of the oxidative phosphorylation. The hypothesis is that IP causes a reversible MPTP opening and a subsequent mitochondrial depolarization which reduces the driving force for Ca<sup>2+</sup> uptake into the matrix which would largely occur during reperfusion after a prolonged ischemic period (Halestrap *et al.* 2007; Hausenloy *et al.* 2009; Zorov *et al.* 2009). During reperfusion following prolonged ischemic period, after IP stimulus, reperfusion injury salvage kinase (RISK) program is activated. It determines the parallel activation of the PI3K/Akt and the extracellular regulated kinase system with downstream p70 ribosomal protein S6 kinase (p70S6K) and glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) activation leading inhibition of MPTP opening with a reduction of myocardial cell death (Juhaszova *et al.* 2005; Downey *et al.* 2007; Heusch *et al.* 2008).

From this mechanism it is clear that the “priming” of MPTP during IP stimulus represent the “hormetic” mechanism by which IP is able to inhibit the large MPTP opening during ischemia-reperfusion injury which represents the key point of cell survival.

## **AGE-RELATED REDUCTION OF IP**

Age-related reduction of IP was firstly obtained in the isolated and perfused heart model in which a short period of ischemia (2 minutes) followed by 10 minutes reperfusion before a prolonged ischemic period (20 minutes) and reperfusion (40 minutes), was able to induce an improvement of left ventricular function in hearts from adult (6 months) but not in those from senescent rats (24 months) (Abete *et al.* 1996). Moreover, the age-related reduction of IP has been confirmed in several studies (Tani *et al.* 1997; Fenton *et al.* 2000; Bartling *et al.* 2003; Boengler *et al.* 2007).

This phenomenon has been successively confirmed in clinical studies. Preinfarction angina, the most evident equivalent of IP, is able to reduce in-hospital mortality in adult but not in elderly patients (Abete *et al.* 1997): this result was confirmed by multivariate analysis showing that preinfarction angina is protective against mortality only in adult patients (Abete *et al.* 1997). The same result was successively confirmed at 5-years follow-up (Ishihara *et al.* 2000). Both dynamic electrocardiography (Napoli *et al.* 1999) and effort exercise (Longobardi *et al.* 2000) have demonstrated that “warm-up phenomenon” is reduced in elderly patients. Finally, the age-related reduction of IP has been also demonstrated in elderly patients undergoing coronary angioplasty (Lee *et al.* 2002) and coronary bypass (Wu *et al.* 2001).

## **HYPOTHETICAL MECHANISM OF AGE-RELATED IP REDUCTION**

Age-related decline of catecholamines release due to several mechanisms, including a related diminished ability for catecholamine synthesis, has been described (Mazzeo and Horvarth, 1987; Dawson and Meldrum, 1992). Accordingly, it has been shown that norepinephrine release from coronary effluent was reduced in senescent hearts in response to IP stimulus (Abete *et al.* 1996). PKC translocation in response to IP is also impaired in aging heart and it probably is responsible for the loss of age-related reduction of IP cardioprotection (Tani *et al.* 2001). Moreover, p38 MAPK and HSP27 have been demonstrated failing to activate in senescent IP model (Peart *et al.* 2007). Finally, because an age-related up-regulation of protein phosphatase 2A, an enhanced dephosphorylation by protein phosphatases has been considered another mechanism of age-related IP reduction (Fenton *et al.* 2005).

## **RE-ESTABLISHMENT OF IP IN THE AGING HEART**

Restoring and/or preventing the age-related IP reduction by means of drugs, acting at different steps of IP mechanism, has been experienced. More importantly, anti-aging interventions, such as exercise training and caloric restriction have been tested (Pepe, 2001; Jahangir *et al.*

2007). Adenosine (McCully *et al.* 1998) and the inhibition of phosphatase (Fenton *et al.* 2005) are able to restore IP in different senescent experimental model. Interestingly, the effect of IP on developed pressure recovery was absent in sedentary but present in trained senescent hearts (Abete *et al.* 2000) and in food-restricted senescent animals (Abete *et al.* 2002). However, the efficacy of physical activity and caloric restriction was only partial while it was complete in hearts from combined trained and food restricted senescent rats (Abete *et al.* 2005). The approach in restoring age-related IP reduction is more difficult in humans for ethical reasons. In a very important study in elderly patients undergoing coronary angioplasty the IP impairment was reversed by nicorandil administration, a specific agonist of mitochondrial  $K_{ATP}$  channels and its effect was inhibited by the proglibenclamide, a blocker of  $K_{ATP}$  channels (Lee *et al.* 2002). According to animal studies, preinfarction angina, IP clinical equivalent, was able to reduce mortality in elderly patients only in those with a high level of physical activity (Abete *et al.* 2001) as well as in those with normal body-mass index (BMI) (Abete *et al.* 2003). Similar to that observed in animal studies, the effect of physical activity and normal BMI was synergistic. In fact, preinfarction angina reached the maximum protective effect at highest PASE and lowest BMI score. Interestingly preinfarction angina is predictive of mortality in “sedentary overweight” but it is “protective” in “trained normal weight” elderly patients (Abete *et al.* 2009).

## SUMMARY

Cardiac IP represents the most powerful endogenous protective mechanism against ischemia through brief episodes of ischemia which are able to protect the heart against a successive more prolonged ischemic period. IP represent a classical example of “hormesis”. However, this powerful protective mechanism is reduced with aging both in experimental and clinical studies. Alterations of mediators release and/or intracellular pathways may be responsible for age-related ischemic preconditioning reduction. Exercise training and caloric restriction separately, and more powerfully taken together, are able to completely preserve and/or restore the age-related reduction of ischemic preconditioning. Interestingly, adenosine and nicorandil, an opener of mitochondrial  $K_{ATP}$  channels, are able to restore IP in animal and human senescent models. However, this kind of hormesis is still not ready for application to humans right now. But, since the complex mechanism of IP is completely elucidated, development of animal and human studies designed to preserve the efficiency of this cardioprotective mechanism in the aged heart are required.

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