INTRODUCTION

Coronary artery disease (CAD) or ischemic heart disease is related to the stenosis of the coronary artery along with the arteriosclerosis. Sudden reperfusion of an ischemic heart induces a series of adverse events resulting in myocardial damage called as ischemia-reperfusion injury (I/R injury) (Collard, Gelman, 2001; Kloner, 1993). CAD is the leading cause of mortality in industrialised countries, and the major risk factors include family history, lack of exercise, obesity, diabetes, smoking, high blood pressure, and mental stress. Treatment can be done through percutaneous transluminal coronary angioplasty, cardiac valve replacement, and bypass-grafting of coronary artery and each of them could be treated according to the extent and health of the patients (Go et al., 2013). Despite improved surgery, ischemia and reperfusion remain a major cause of myocardial injury during cardiac surgery (Liu et al., 2012; Marczak et al., 2012). Reperfusion is necessary for the recovery of ischemic myocardium from infarction. Still, it also leads to irreversible myocardial damage, and thus the protection of the myocardium from ischemia-reperfusion injury during surgery remains significant (Han et al., 2013). Ischemic preconditioning (IPC) is one of the most effective ways of protecting the myocardium from ischemic attacks by various pathways (Murry, Jennings, Reimer, 1986; Snoeckx et al., 1993; Ferdinandy, Schulz, Baxter, 2007; Marina Prendes et al., 2007). However, the shielding effect of IPC has been proven to be assuaged under certain pathological conditions like hypertension,....

Role of caveolin-eNOS platform and mitochondrial ATP-sensitive potassium channel in abrogated cardioprotective effect of ischemic preconditioning in postmenopausal women

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Caveolin, the protein of the caveolar membrane, interacts and binds with endothelial nitric oxide synthase (eNOS), forming a caveolin-eNOS complex leading to suppression of the eNOS activity. Caveolin, therefore, maintains eNOS in the inactivated state leading to reduced nitric oxide (NO) production. Ischemic preconditioning disrupts the caveolin-eNOS complex leading to activation of the eNOS and thus results in cardioprotection. During ischemic preconditioning, NO produces cardioprotection by the opening of the $K_{ATP}$ channel, and the caveolin forms a suitable signalling platform facilitating the interaction of NO with the $K_{ATP}$ channel. Estrogen deficiency has been reported to upregulate caveolin-1 expression. The article aims to review the various mechanisms that placed the women at the risk of coronary artery diseases after postmenopausal estrogen deficiency and their role in the cardioprotective effect of ischemic preconditioning.

Keywords: Caveolin. Nitric oxide. Mito $K_{ATP}$. Ischemic preconditioning. Postmenopause.

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hyperlipidaemia, diabetes, aging and heart failure (Snoeckx et al., 1986; Abete et al., 1996; Ferdinandy, Szilvassy, Baxter, 1998; Yadav, Singh, Sharma, 2010a; 2010b; Ajmani et al., 2011). Interestingly, it has been seen that the likelihood of the incidence of CAD is higher in men than in women. Nevertheless, the incidence of CAD in women after menopause is the same as in men of the same age (Barrett-Connor, 1997; Clarkson et al., 1997). Therefore, in this review, we are focusing on the mechanisms responsible for putting the women at the risk of CAD after postmenopausal estrogen deficiency and their involvement in the cardioprotective effect of IPC.

**METHODS**

Appropriate studies were collected through Pubmed, Medline, Scopus, Google Scholar online searches. The terms “ischemia-reperfusion,” “ischemia-reperfusion injury,” or “ischemic preconditioning,” along with “nitric oxide,” “mito K_{ATP},” “caveolin,” “postmenopause,” “ovariectomized,” were used for searching. Besides, we looked for the bibliographies of relevant studies, reports, and editorial letters for writing this review.

**Ischemic reperfusion injury**

Myocardial ischemia occurs when the blood supply to the heart is inadequate (Gasser et al., 1994). Early restoration of blood flow, i.e., reperfusion, is necessary for the survival of ischemic heart (Anaya-Prado, 2002). However, reperfusion after a prolonged period of ischemia itself can elicit a cascade of adverse events that paradoxically causes tissue injury that is called I/R injury (Kloner, 1993; Collard, Gelman, 2001). Ischemia-reperfusion injury leads to myocardial stunning and microvascular injury, which leads to necrosis of myocardium (Ambrosio, Titto, 1999; Yellon, Baxter, 2000).

During ischemia as indicated in Figure 1, there is reduced oxidative phosphorylation, decreased ATP level, and subsequent increase in the concentration of ADP, AMP, and phosphate (Solaini, Harris, 2005; Powers et al., 2007). The decrease in ATP activates anaerobic respiration resulting in the reduction of intracellular pH and activation of Na⁺/H⁺ antiporter (Buja, 2005). The Na⁺ that enters this route is normally pumped via Na⁺/K⁺ ATPase. Still, decreased ATP inhibits this efflux leading to the gradual rise in intracellular Na⁺ and subsequent increase in the concentration of intracellular calcium ions (Piper, Abdallah, Schäfer, 2004).

**FIGURE 1 - Ischemic Reperfusion Injury.**
AMP is converted to adenosine, which gets further converted into inosine and to hypoxanthine (Szocs, 2004). During reperfusion, hypoxanthine is oxidised by xanthine oxidase, which produces reactive oxygen species (ROS). Ischemia-reperfusion leads to the production of ROS from the mitochondria (Detmers et al., 1999; Elimadi et al., 2001; Becker, 2004) which is reported to damage the cell membranes by lipid peroxidation (Halestrap, Clarke, Javadov, 2004; Halestrap, 2006; Solaini, Harris, 2005). Moreover, in the first few minutes of reperfusion, oxidising agents such as superoxide anion, hydroxyl radical, and peroxynitrite are generated that cause marked damage to the myocardium (Bolli et al., 1989). Ca++ and elevated level of cytosolic ROS is known to open the mitochondrial permeability transition pore (mPTP) (Powers et al., 2007; Baines, 2009). mPTP are multiprotein complexes that form non-selective pores in the inner mitochondrial membrane (Powers et al., 2007, Baines, 2009) and their opening leads to release of cytochrome C into the cytoplasm and initiates the process of apoptosis through caspase 9 (Cardone et al., 1998) and caspase 3 (Zou et al., 1997; Weiland et al., 2000).

The opening of mPTP causes depolarisation of the inner mitochondrial membrane resulting in a decrease in ATP production, and even stored ATP gets consumed to maintain inner mitochondrial membrane potential (Honda, Korge, Weiss, 2005). Depletion of ATP and elevated Ca++ during ischemic insult activate the degradative enzymes such as phospholipases (PLA2) (Ford, 2002) and calcium-activated proteases (calpains) (Chen et al., 2002) with inhibition of ATP-dependent cytosolic repair processes due to lack of ATP, eventually resulting in the loss of cellular integrity (Murphy, Steenbergen, 2008).

It has been reported that persistently elevated level of calcium and ROS is responsible for membrane disruption, massive cell swelling, cell lysis (Haussenloy, Yellon, 2004) and ultimately contribute to necrotic cell death (Zong, Thompson, 2006). Necrosis results in rapid loss of plasma membrane integrity due to increased oxidative stress, cytosolic calcium level, and decreased level of ATP (Ermak, Davies, 2002; Bartosz, 2009).

Moreover, I/R injury has been well demonstrated to cause organ damage in the brain, heart, lungs, liver, kidneys, and skeletal muscle (Novgorodov, Gudz, 2009). Several therapeutic strategies such as controlled reperfusion, preconditioning, postconditioning, and several pharmacological interventions, for example, adenosine (Lozza et al., 1997; Moukarbel, Ayoub, Abchee, 2004), renin-angiotensin system antagonist (Paz et al., 1998), calcium antagonists (Segawa et al., 2000), antioxidants (Marczin et al., 2003), sodium-hydrogen exchange inhibitors (Hennan et al., 2006), iron chelators (Tang et al., 2008), N-methylated synthetic sphingolipid analog (Gundewar, Lefer, 2008), flavonoids (Yadav et al., 2015) and exenatide (Timmers et al., 2009) have shown to reduce ischemia-reperfusion-induced myocardial injury.

**Concept of preconditioning**

In 1986, Murry and co-workers provided the strategy to prevent I/R injury. They found that short transient periods of sublethal ischemia accompanied by reperfusion protect the myocardial tissue from prolonged ischemic insult, which is known as “Ischemic preconditioning” (IPC) (Murry, Jennings, Reimer, 1986; Tomai et al., 1999). This potent cardioprotective strategy has been observed in all animal species examined to date, including mammals (Cohen, Liu, Downey, 1991). Ischemic preconditioning is a biphasic process, an early phase that begins within minutes and slowly decreases within 2-3 hours and called classical preconditioning (Downey, Cohen, 1997; Yellon, Downey, 2003). The other is a late phase that occurs after 12-24 hours of ischemic insult and lasts 3-4 days and called as late phase preconditioning or second window of protection (Kuzuya et al., 1993; Marber et al., 1993). The early phase IPC only protects from myocardial infarction, but the late phase IPC also protects from myocardial stunning (Bolli, 1996; Sisakiyan, 2008).

Many pharmacological agents have been shown a preconditioning-like effect, i.e., adenosine (Liu et al., 1991; Yao, Gross, 1994), bradykinin (Goto et al., 1995; Yoshida et al., 2005), protein kinase C activators (Ytrehus, Liu, Downey, 1994), ATP sensitive potassium channel openers (Parratt, Kane, 1994; Schulz, Rose, Heusch, 1994), opioids (Schultz et al., 1995), norepinephrine (Thornton et al., 1993), acetylcholine (Yao, Gross, 1993), α1 adrenergic receptors agonists (Banerjee et al., 1993),
They bind to their respective G-protein coupled receptors and initiates a cascade of signal transduction, which leads to activation of PI3K (Mocanu et al., 2002) and phospholipase C (Tyagi, Tayal, 2002). Activated...
PI3K generates phosphatidylinositol 3,4,5-triphosphate (PIP3) from cell membrane lipid phosphatidylinositol 3,4-bisphosphate (PIP2) leading to activation of the phosphoinositide-dependent kinase (PDK1) and subsequent activation of protein kinase B (Akt) and p70S6-kinase (Jonassen, Mjos, Sack, 2004; Kis, Yellon, Baxter, 2003). PI3K activation reported to upstream of PKC (Tong et al., 2000), GSK3β (Tong et al., 2002), and activation of mitochondrial ATP-sensitive K channels (mito K_{ATP}) (Oldenburg et al., 2002; Garlid et al., 1997). The activated phospholipase C leads to the generation of two-second messengers, diacylglycerol (DAG) and inositol triphosphate (IP3), by hydrolysis of PIP2. The DAG activates protein kinase C by translocating it from cytosol to perinuclear membrane (Mitchell et al., 1995; Tong et al., 2004). ROS generation during preconditioning also activates PKC (Penna et al., 2009; Baines, Goto, Downey, 1997). PKC activation is important in the opening of mito K_{ATP} (Sato, O’Rourke, Marban, 1998; Murphy, 2004). PKC_{ε}, as well as PKCδ, has been demonstrated to mimic preconditioning due to the opening of mito K_{ATP} (Dreixler et al., 2008).

The opening of mito K_{ATP} channels can protect the mitochondria from Ca^{2+} overload and prevent cytochrome c loss (Garlid et al., 1997; Korge, Honda, Weiss, 2002). As potassium enters the mitochondria, it causes them to release free radicals, i.e., ROS (Downey, Cohen, 2006). Although a massive burst of ROS leads to cell damage, a moderate release of ROS during nonlethal short episodes of ischemia play a significant triggering role in the signal transduction pathways of IPC (Vanden et al., 1998). PKC_{ε} also forms a complex with mitochondrial permeability transition pore (mPTP) (Baines et al., 2003; Zoratti et al., 2009), which leads to decrease in the release of cytochrome C and apoptotic cell death (Kroemer, Dallaporta, Resche-Rigon, 1998; Hausenloy, Yellon, 2004).

Clinical aspects of ischemic preconditioning

Numerous studies have been well demonstrated the clinical potential of preconditioning in patients of ischemic heart disease. Various in vivo models of ischemic preconditioning in human myocardium have been shown including warm-up phenomenon, preinfarction angina, angioplasty studies, and other surgical studies (Yellon, Downey, 2003). The ischemic preconditioning phenomenon was well demonstrated in the human atrial muscle of patients undergoing coronary artery bypass graft surgery (CABG) (Walker et al., 1994). Other invitro studies also indicated that the δ-opioid receptor as a trigger in human myocardium subjected to ischemic preconditioning (Bell et al., 2000). Myocardial biopsies were taken after 10min of cross-clamping exhibited significantly higher content of ATP and reduced release of troponin (Tomai et al., 1999; Ylitalo, Peuhkurinen, 2000). Pharmacological recruitment of protection using adenosine (Mentzer et al., 1997), volatile anesthetics, i.e., isoflurane (Belhomme et al., 1999; Riess, Stowe, Wartlir, 2004; Frassdorf et al., 2009) is another interesting alternative to provide preconditioning mediated cardioprotection in patients undergoing CABG (Tomai et al., 1999, Ylitalo, Peuhkurinen, 2000).

The Post-transluminal coronary angioplasty (PTCA) procedure involves repeated intracoronary balloon inflations with intervening periods of perfusion which was characterized by less anginal pain, less ST-segment shift, and lower mean pulmonary artery pressure, despite a reduction in cardiac vein flow and unchanged coronary wedge pressure during second balloon inflation (Yellon, Downey, 2003). Pre-treatment with Nicorandil, a mito K_{ATP} channel opener preconditions the myocardium by preventing the incidence of ventricular arrhythmias and myocardial dysfunction after coronary reperfusion (Kato et al., 2001).

Further, adenosine preconditioning decreases the severity of ischemia during the first balloon inflation, and that was significantly improved on subsequent balloon inflations during PTCA (Leesaret et al., 2003). The warm-up phenomenon improves coronary blood flow and reduced myocardium oxygen consumption during the second period of exertion (Okazaki et al., 1993; Marber, Joy, Yellon, 1994). This endogenous adaptation has been studied during successive ergometer or walking tests and during repeated atrial and ventricular pacings (Joy, Cairns, Springings, 1987; Ylitalo, Peuhkurinen, 2000; Ylitalo et al., 2001). Patients with pre-infarct angina were found to have smaller creatine kinase output, less
arrhythmias, less stunning and heart failure and better in-hospital outcome after thrombolytic therapy than patients without pre-infarction angina (Anzai et al., 1995; Andreotti et al., 1996; Kloner et al., 1998; Skyschally et al., 2005; Yan et al., 2009). Pre-infarct angina may activate endogenous antithrombotic or fibrinolytic mechanisms, which gives more time for revascularization procedures (Haider et al., 1995; Tomoda, Aoki, 1999).

The findings from many preclinical studies in which cardioprotection has been seen in healthy animal hearts might not be reproducible in the human myocardium due to several factors such as old age, the presence of comorbid disease such as diabetes, hypertension, hypercholesterolemia (Goyal, Agrawal, 2017; Varshney et al., 2017). Moreover, the timing and duration of myocardial ischemia, use of pharmacological agents such as oral sulfonylurea drugs or cyclooxygenase 2 inhibitors and practical constraints may complicate preconditioning protocol and limit the benefits of these drugs under such clinical conditions (Schulman, Latchman, Yellon, 2001; Riess, Stowe, Wartlir, 2004).

Role of nitric oxide (NO) in preconditioning

It has been demonstrated that NO is involved in preconditioning induced PKCε translocation (Ping et al., 1999). Because inhibition of PI3K leads to the reduction in the generation of NO, it can be concluded that PI3K activates PKCε via eNOS mediated mechanism (Tong et al., 2000). Akt also directly activates eNOS (Fulton et al., 1999; Dimmeler et al., 1999), and NO generated by eNOS is proposed to initiate preconditioning (Ping et al., 1999). It has been demonstrated that NO generated during preconditioning is a trigger for late PC (Ping et al., 1999), but the role of NO in early PC is controversial (Woolfson et al., 1995). The mechanism by which NO activates PKCε is still to be elucidated. Because the antioxidant mercaptopropionyl glycine blocks NO-donor induced late PC (Takano et al., 1998), it can be postulated that NO-derived reactive species (ONOO⁻) may activate PKCε either by direct oxidative modification or via activation of phospholipases (Ping et al., 1999). eNOS generates NO, which results in activation of guanylyl cyclase, which via protein kinase G is reported to activate a mitochondrial PKCε, which results in the opening of the mito K_ATP channel (Costa et al., 2005).

Biology of caveolae

The term Caveolae was coined by Yamada in 1955 to reflect their appearance as “little caves”, which is 50-100 nm in diameter (Roth, Porter, 1964). Caveolae are plasma membrane invaginations on the surface of endothelial cells (Palade, 1953). Glenney in 1989 first identified caveolin as a 21-22KDa tyrosine-phosphorylated substrate in chick fibroblasts. Caveolae are the specialized membrane domains, triton insoluble, cholesterol and sphingolipids enriched protein (Garcia-Cardena et al., 1997) which form lipid raft with caveolins (Williams, Lisanti, 2004) that serves as organizing centers for cellular signal transduction (Shaul, Anderson, 1998; Patel, Murray, Insel, 2008). Caveolin also possesses a scaffolding domain that facilitates the interaction and organization of signaling molecules to provide coordinated and efficient signal transduction (Okamoto et al., 1998).

The caveolin gene family consists of three members that differ in their pattern of expression in different cell types. Caveolin-1 (cav-1) and caveolin-2 (cav-2) are co-expressed in many cell types including adipocytes, endothelial cells, epithelial cells and fibroblast (Scherer et al., 1994; Scherer et al., 1997) whereas Caveolin-3 (cav-3) is restricted to the skeleton and smooth muscles including cardiac myocytes (Scherer et al., 1994; Song et al., 1996; Minetti et al., 1998; Galbiati et al., 2001). It is also found in a variety of other cells, including the immune and nervous system. Cav-1 is a specific marker of caveolae and is up-regulated by oxidized LDL, estrogen deficiency, and hyperglycemia (Sharma, Singh, Sharma, 2011). It serves as a cholesterol-binding protein and helps cholesterol to move from endoplasmic reticulum through the golgi apparatus to the plasma membrane of endothelial cells (Fulton, Gratton, Sessa, 2001). Caveolin is a negative regulator of eNOS as its interaction, and binding suppresses the activity of eNOS by making a caveolin-eNOS complex (Minshall et al., 2002; Feron, Balligand, 2006; Koneru et al., 2007). Alterations in caveolin/eNOS interaction influence various mechanisms of diseases such as atherosclerosis, diabetes, cirrhosis.
(Spieker, Lüscher, Noll, 2001; Elçioglu et al., 2010; Xu et al., 2008; Ajmani et al., 2011).

Various signaling molecules have been shown to localize within caveolae. These include SCR family, tyrosine kinase, GPCR, members of Ras-MAPK cascade, and nitric oxide synthase (Ostrom, Insel, 2004; Insel et al., 2005). It has been documented that phosphatidylinositol-3 kinase/protein kinase B (PI-3K/AKT) pathway, PKC and PKA in caveolae interact with caveolin and modulate the opening of ATP-dependent K+ channels and regulate the survival of cell facilitating the interaction of NO with K<sub>ATP</sub> channel by forming a suitable signaling platform (Razani, Lisanti, 2001). Caveolins (cav-1 and cav-3) maintains eNOS in the inactivated state, which leads to a decrease in NO production (Quinlan et al., 2008; Garcia-Cardena et al., 1997; Maniatis et al., 2006). Increased disruption of the caveolin/eNOS complex by calcium/calmodulin-binding to eNOS leads to an increase in the activity of eNOS (Feron, Balligand, 2006). It has been reported that activation of PKA and Ras-p42/44 MAPK downregulates cav-1 expression (Engelman et al., 1999). Moreover, overexpression of cav-3 in myocardium has been reported to mimic preconditioning by activating PI3-K (Tsutsumi et al., 2008).

**Role of caveolin in ischemic preconditioning**

It has been documented that there is the involvement of caveolins and caveolae in protecting the heart from ischemia/reperfusion injury (Gratton, Bernatchez, Sessa, 2004; Ajmani et al., 2011). Caveolae disruption in cardiac myocytes abolished cardiac protection (Patel et al., 2007). Signaling molecules, as shown in Figure 3 involved in cardiac protection, include GPCRs and the protein tyrosine kinase Src, which compartmentalize within caveolae and interact with the scaffolding domain of caveolin (Krajewska, Masłowska, 2004). Various GPCRs such as opioids and adenosine promote cardiac protection as well as post-receptor components that can enhance protection localize to caveolae and co-immunoprecipitate with caveolins (Head et al., 2005). Further, it has been reported that the infusion of the caveolin scaffolding domain (CSD) peptide of cav-1 into ischemic/reperfused hearts results in the recovery of cardiac function (Young, Ikeda, Lefer, 2001). Further, treatment with isoflurane modifies cardiac myocytes sarcolemmal structure and composition leads to activation of Src kinase and phosphorylation of cav-1 contributes to cardiac protection (Patel et al., 2007). It is documented that calmodulin disrupts the heterotrimeric complex formed between eNOS and caveolin in a calcium-dependent manner (Michel et al., 1997).

Moreover, caveolin (cav-1 and cav-3) maintains eNOS in inactivated state and thereby limits NO production (Maniatis et al., 2006), but agonist stimulation leads to activation of eNOS through the increase in calcium and disruption of caveolin/eNOS heterocomplex (Feron, Balligand, 2006). It has been reported that activation of PKA and Ras-p42/44 MAPK downregulates cav-1 expression (Engelman et al., 1999). Moreover, overexpression of cav-3 in myocardium has been reported to mimic preconditioning by activating PI3-K (Tsutsumi et al., 2008). Ischemic preconditioning induces the translocation of eNOS and GLUT-4 to and from the plasma membrane, which is essential for cardioprotection (Koneru et al., 2007). However, preconditioning with angiotensin II improves post-ischemic ventricular recovery, reduces myocardial infarction and decreases cardiomyocyte apoptosis (Das, Das, Das, 2007) which is
due to decrease association of p38MAPKβ and ERK1/2, i.e., anti-death signalling components with caveolin and increased association with p38MAPKα and JNK, i.e., death signaling components generate survival signal as demonstrated by increased phosphorylation of Akt and enhanced induction of expression of Bel-2 in the heart (Das, Das, Das, 2007). Moreover, Pharmacological Preconditioning with bradykinin induces the formation of a caveolar signaling platform (signalosomes) that contains the enzymes of the signaling pathway which interact with mitochondria to induce the opening of mito $K_{\text{ATP}}$ channel (Quinlan et al., 2008).

**Mitochondrial $K_{\text{ATP}}$ and ischemic preconditioning**

ATP-Sensitive $K^+$ Channel was first identified in 1983 in the myocardium (Noma, 1983). Two subtypes of $K_{\text{ATP}}$ channels have been documented, one is sarcolemmal $K_{\text{ATP}}$ channel (sarc $K_{\text{ATP}}$) which is present on the cell membrane (Aguilar-Bryan et al., 1998) while other is located in the inner membrane of the mitochondria and known as mitochondrial ATP sensitive potassium channels (mito $K_{\text{ATP}}$) (Yokoshiki et al., 1998). The $K_{\text{ATP}}$ channels belong to the ATP-binding cassette transporter superfamily. Sarcolemmal $K_{\text{ATP}}$ channel is composed of an octameric complex of two types of subunits, the Kir6.2 and the SUR2A subunit whereas mito $K_{\text{ATP}}$ channel is comprised of two subunits one is a pore-forming, inward-rectifying potassium channel subunit (Kir), and other is regulatory sulfonylurea receptor (SUR) (McCully, Levitsky, 2003; Mironova et al., 2004).

Mito $K_{\text{ATP}}$ channel acts as the trigger, as well as the end effector of ischemic preconditioning mediated cardioprotection (Gross, Peart, 2003). Moreover, the opening of mito $K_{\text{ATP}}$ channel leads to an influx of $K^+$ in the mitochondrial matrix (da Silva et al., 2003) resulting in depolarised inner mitochondrial membrane and reduced mitochondrial calcium entry into the mitochondria resulting in inhibition of opening of mPTP (Costa et al., 2006; Zoratti et al., 2009) consequently decrease in the release of cytochrome C and reduction of apoptotic cell death (Kroemer, Dallaporta, Resche-Rigon, 1998; Javadov et al., 2003; Hauserloy, Duchen, Yellon, 2003; Hauserloy, Yellon, 2004). The influx of $K^+$ facilitates the entry of weak acids into the mitochondrial matrix and accelerates the process of oxidative phosphorylation (Tanonaka et al., 1999). In addition, the opening of mito $K_{\text{ATP}}$ channels has been shown to cause partial alkalization, and a small reduction of transmembrane potential leading to the production of ROS (Penna et al., 2007) which mediate the cardioprotective effect of ischemic preconditioning by activation of PKC (Bouwman et al., 2004; Andrukhiv et al., 2006). Several potassium channel openers such as cromakalim (Grover et al., 1995), bimakalim (Puddu et al., 2006), diazoxide (Lawrence et al., 2001; Droese, Brandt, Hanley, 2006), have been reported to produce cardioprotection against ischemia reperfusion-induced injury. A specific blocker of mito $K_{\text{ATP}}$ channel, i.e., 5-hydroxy decanoate (Hide, Thiemermann, 1996; Yang et al., 2009), has been shown to block the protective effects of ischemic preconditioning in the heart. On the other hand, HMR 1098, a specific blocker of sarc $K_{\text{ATP}}$ channel, has been demonstrated to abolish the protective effects of ischemic preconditioning (Suzuki et al., 2002).

**Ischemic preconditioning in postmenopausal heart**

It has been reported that men are more susceptible than women to hypertension and cardiovascular diseases (Barrett-Connor, 1997). However, after menopause in women, the risk of ischemic heart disease reaches to the same level as in men of the same age (Clarkson et al., 1997; Barrett-Connor, 1997), which indicates that the female sex hormones, particularly estrogen plays a crucial role in reducing the risk of ischemic heart diseases (Sullivan et al., 1998; Stampfer, 1995). The dramatic increase in the ischemic heart disease is the leading cause of death in postmenopausal women (Bush et al., 1988) and the estrogen replacement therapy lowers the incidence of cardiovascular events (Stampfer et al., 1985; Bush et al., 1987). However, several clinical studies failed to demonstrate any cardioprotection from such estrogen replacement therapy (Barrett-Connor, Stuenk el, 1999; Rossouw et al., 2002). Rossouw et al., 2002 has been reported that the incidence of ischemic heart disease was increased in women receiving estrogen as compared to those receiving placebo.

Cardiomyocytes from female hearts are more resistant to ischemia-reperfusion-induced injury as a
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Comparison of male hearts (Ranki et al., 2001). It has been documented that an increased level of phosphorylated Akt and PKCε are responsible for cardioprotection against I-R induced injury in female hearts (Bae, Zhang, 2005).

Estrogen deficiency is associated with increased TNF-α level, which may lead to increased myocardial injury after menopause (Liao, Chen, Chen, 2002). In another study, it has been reported that decreased mitochondrial respiration and increased mPTP opening with aging are responsible for necrotic cell death associated with ischemia/reperfusion injury in postmenopausal women (Machikas et al., 2018).

Shinmura and coworkers demonstrated that the cardioprotective effect of IPC is lost in ovariectomized rats, which is partly due to impaired phosphorylation and translocation of PKCε to the membrane. Moreover, estrogen replacement or selective activation of PKCε-mediated signaling restores the cardioprotective effect of IPC (Shinmura et al., 2008).

Montalcini et al. (2007) found that the development of cardiovascular diseases after menopause is not only due to a decrease in estrogen but also due to a decrease in androgen. Furthermore, it has been reported that testosterone enhances estradiol’s cardioprotection in ovariectomized rats, estradiol and testosterone combination protects cardiomyocytes against I-R injury (Liu et al., 2011). It has been well documented that ovariectomy (surgical menopause) reduces the protein expression of eNOS and increases the cav-1 expression subsequently decrease the activation of mito KATP channels in cardiac tissue which is also the main cause of abrogated cardioprotective effect of IPC (Figure 4; Pelligrino et al., 2000; Goyal, Semwal, Yadav, 2016) but the chronic estrogen treatment or phytoestrogens like daidzein accompanies restoration of the normal activity of myocardial eNOS (Wang et al., 2002; Goyal, Semwal, Yadav, 2016).

**FIGURE 4** - Role of Caveolin-eNOS and mito-KATP in Normal and Estrogen Deficient Condition.
Endogenous and exogenous estrogen in premenopausal and postmenopausal women, respectively, protects against cardiovascular disease (Stampfer et al., 1991; Grady et al., 1992). Estrogen acts as a vasoprotective molecule by increasing the bioavailability of nitric oxide (Best et al., 1998; Levin, 2005). Estrogen upregulates eNOS and downregulates its inhibitory protein cav-1 (Hishikawa et al., 1995). The cardioprotective effects of estrogen are, in part, mediated by the regulation of TNFα levels in the ischemic heart (Xu et al., 2006). The effect of estrogen on eNOS expression is mediated via estrogen receptors α (ERα) and β (ERβ), which are present on endothelial cells (Gavin et al., 2009).

Activation of eNOS by estrogen has been reported to occur through ERK-1/2 (Chen et al., 1999) pathway as well as via the phosphatidylinositol 3-kinase (PI3K)/protein kinase (Akt) pathway (Simoncini et al., 2000; Hisamoto et al., 2001; Haynes et al., 2000). The recruitment of the latter cascade depends on the ligand-dependent association of ERα with PI3K (Simoncini et al., 2000). Akt can be activated by estrogen (Camper-Kirby et al., 2001), which further activates eNOS by phosphorylating it at serine 1177 residue (Fulton et al., 1999; Dimmeler et al., 1999). This phosphorylation not only activates eNOS but also increases the efficiency of activation by Ca++/calmodulin (McCabe et al., 2000). Thus, estrogen increases the bioavailability of NO and thus results in a decrease in myocardial injury. In addition, 17β-estradiol has been shown to reduce myocardial necrosis in rabbits after ischemia and reperfusion (Hale, Birnbaum, Kloner, 1996) and improve recovery of mechanical function following global ischemia in isolated rat hearts (Kolodgie et al., 1997; Fraser et al., 1999).

CONCLUSION

The cardioprotective potential of IPC is lost in estrogen deficiency. In this condition, the outcome of I/R injury worsens, and the infarct size limiting effect of IPC is blunted due to the upregulation of caveolin and downregulation of nitric oxide as well as inhibition of mito K$_{ATP}$ channel. This may affect the clinical application of IPC in patients with estrogen deficiency or postmenopausal women undergoing cardiac surgery. Therefore, we can say that by adopting the approaches like inhibiting caveolin, upregulating nitric oxide, and the opening of mito K$_{ATP}$ can help in regaining the cardioprotective effect of IPC in postmenopausal or estrogen-deficient condition.

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All authors have no conflict of interest to declare.

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