Introduction

According to the mortality rate data, in 2007, more than 2200 Americans die of cardiovascular disease each day, giving an average of 1 death every 39 seconds [1]. During the same year, an estimated 1 of every 6 deaths were caused by coronary heart disease. The recent statistics published in Circulation review indicate that approximately every 25 seconds, an American will have a coronary event, approximately every minute, someone will die of one and on average, every 40 seconds, someone in the United States has a stroke [2]. With this rate, cardiovascular diseases are the leading cause of death in the US after cancer. Ischemic myocardial injury occurs in many clinical conditions such as heart transplantation, cardiac bypass, and coronary stenting after acute myocardial infarction. Understanding the mechanisms occurring during an ischemia/reperfusion sequence to protect patient against damage caused by this events has become a public health problem. In the clinic, drug administration to patient is the principal approach used to help patient to better recovery. Despite, encouraging results obtained in the past, for example from 1998 to 2008, the stroke death rate fell 34.8%, and the actual number of stroke deaths declined 19.4% according to the American Heart Association, in conjunction with the Centers for Disease Control and Prevention, the National Institutes of Health report [3]. Cardiovascular diseases continue to be a big preoccupation of health care authorities in US. The development of new approaches, including new techniques as well as the discovery of novel molecules could provide means for new ways of heart protection, new opportunities to better treat cardiovascular complications. To this aim, several pre-clinical studies have proposed new technique to protect the heart against ischemic myocardial injury. In 1986, Murry et al came up with the ingenious idea to perform short cycles of ischemia/reperfusion before a prolonged ischemic insult and found that this maneuver...
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attenuated myocardial infarct size [4]. Several studies have been performed to elucidate the mechanisms of this powerful method [5,6]. However, the clinical application of this technique has been rather difficult because administration of drug at the onset of the ischemia is clinically impractical. It becomes more than necessary to protect the ischemic myocardium during the reperfusion. This alternative approach is more relevant and clinically practical. Two promising approaches have been described to protect the ischemic myocardium against injury: The controlled reperfusion [7] and postconditioning [8].

In this paper, we will first gain insight into the physiopathological events and mechanisms occurring during ischemia/reperfusion, introduce and review some general consideration in the use of these two methods of protection as well as critically discuss the clinical relevance of this approach.

Modifications due to ischemia

Ischemia is an inadequate blood flow to a local area of an organ due to blockage of the blood vessels in that area. This inadequate blood supply to the heart may lead to several consequences, which depend on the following conditions of the ischemia: The duration of the occlusion [9], the temperature [10], whether this ischemia is partial or global, and the collateral circulation [11]. Ischemia is the cause of the imbalance between inputs and needs in nutrient and oxygen of the myocardium. The reduction of the oxygen input in the myocardium is the cause of mitochondrial dysfunction, which is responsible to the reduction of ATP production. Shrader et al. observed a reduction of 65% in ATP from the basal value, after 15 minutes ischemia [12]. Similarly, Jones et al. in murine model [13] have observed a significant decrease of ATP (95% of basal value) after 40 minutes ischemia.

During the beginning of ischemia, oxygen is not present and unable to bind to hydrogen and the electron given from the substrates of the electron transfer chain. Due to this effect, the oxidative phosphorylation stops to work. From this moment, there is a transition from aerobic to anaerobic respiration, which is easily detectable by observation of the electrocardiographic modifications [14] and the decrease of the myocardial contractility [15].

In the myocardium, the activated glycogenolysis during the first second of global ischemia is quickly slowed down by the increase of reduced equivalents like NADH and FADH₂ and the decrease of the pH.

The β-oxidation stops quickly during ischemia, inducing the release of free fatty acids, which causes arrhythmias and inhibits the K⁺-ATP synthase in mitochondria [16].

Ionic perturbations also are prevalent during ischemia. Garlick et al. reported that after global ischemia, the intracellular pH decreases and reaches 6.2 after 10 min in rat model [17]. The cause of the decrease in pH during ischemia is not clear. However, Opie et al. suggested that the accumulation of lactic acid and the production of CO₂ from the Krebs cycle might be responsible for this decrease in pH [18].

During ischemia, the intracellular concentration of calcium increases, resulting in acidosis, the decrease of Na⁺/H⁺-ATPase and Ca²⁺-ATPase activity[19]. The intracellular concentration of K⁺ also decreases due to the inhibition of the Na⁺/K⁺-ATPase [20] (Figure 1).

In addition, during ischemia, cell swelling occurs, due to many factors that happen in succession: 1) The accumulation of the metabolites from glycogenolysis (lactates, protons), from the usage of the phosphocreatines (creatine, inorganic phosphates), and the catabolism of the high energy phosphate. The accumulation of these metabolites causes an osmotic charge (120-150 mOsmol/l), which leads to the entry of water into the cells [21]. 2) The inactivation of the Na⁺/K⁺-ATPases due to the lack of ATP [22] is responsible for the increase of Na⁺ in the cells [23].

During global normothermic ischemia, the water in the tissues is stable and its entry into cells is limited. However, during reperfusion, edema may occur due to the unlimited supply of water in the plasma. Figure 1 summarizes different events occurring in cardiomyocytes during ischemia.

Modifications due to the reperfusion

After ischemia, the restoration of perfusion
called “reperfusion” is a way to save the ischemic heart. Reperfusion may reduce the damage due to ischemia if it is performed early on [24]. However, although the reperfusion is beneficial for the survival of the ischemic myocardium, it can induce deleterious effect [25]. In other words, ischemia weakens the myocardium, which worsens during reperfusion due to free radical production, increase in cytosolic calcium concentration, and the return of the pH to normal physiological levels as indicated in Figure 2.

As said above, the pH decreases in the cytosol during ischemia, and returns to normal physiological values at the beginning of reperfusion. Bond et al, 1993 reported a significant increase of cell death concomitants with the return of the pH to physiological value [26]. When the pH decreases, it induces the inhibition of the mitochondrial permeability transition pore (mPTP) opening [27]. The total mPTP opening inhibition becomes when the pH reaches 6.2 [28]. It is probable that with the return of the pH to normal physiological levels during reperfusion, it increases the susceptibility of the mPTP to open and induce cell death. The cellular damage due to the variation in pH during reperfusion is called the “pH paradox” [29].

However, this massive input of the oxygen may cause the increase of ROS production in the mitochondria. If the ability of mitochondria to eliminate this ROS is superior to the amount that is being produced, these free radicals will not be deleterious. But, if the capacity of the mitochondria to eliminate the ROS is inferior to the production, this ROS will be responsible for cell death.

The other event that occurs during the reperfusion is calcium overload. The calcium enters the cell via the exchanger Na+/Ca2+ and the L-type channel [30]. After long ischemia, the cells are not able to restore the calcium homeostasis, causing the calcium overload, which is responsible for: 1) Activation of proteases, lipases, and nucleases, 2) Stiffening of the myofibrils of the contractile apparatus, 3) The opening of the mPT pore.

Stone et al. 1989 observed the decrease in post-ischemic injury and the improvement of cardiac functional recovery using the red of ruthenium, an inhibitor of the calcium uniporter. This indicates the involvement of this channel in calcium homeostasis [31].

In 1993, Kloner et al [32] reported the following events which are directly related to reperfusion after long period of ischemia: arrhythmias due to free radical [33, 34], endothelium dysfunction caused by the phenomenon of “no reflow” [35], and the myocardial stunning de-
Controversy about the cell death specific to reperfusion

For many years, several authors thought that reperfusion has only a beneficial effect on the ischemic myocardium [37, 34, 38]. According to these authors, there was no cell death related to myocardium reperfusion. However, others authors have shown that hearts in which cell death by apoptosis is programmed during ischemia will die during reperfusion [39]. In this theory, the reperfusion only accelerates the death of already irreversibly damaged cardiomyocytes during ischemia, but does not induce death of the cells still viable.

The concept of “reperfusion injury” differ to this above description by the fact that reperfusion by itself may be able to induce death to cells that have survived through ischemia, and eventually any cell in which death is programmed. One of the principal reason of the controversy resides in the fact that it is impossible to estimate the own effects of reperfusion. Recently, Zhao et al. showed that performing three cycles of thirty second I/R at the onset of the reperfusion induces cardioprotection effect. This amazing discovery of Vitten-Johansen team has marked the end of the first theory and at the same time confirmed the existence of a so-called “reperfusion injury” [40].

Protection of myocardium during the reperfusion

Reperfusion is a way to save the ischemic myocardium. However, as indicated above, experimental work has indicated an adverse effect of reperfusion on the ischemic myocardium known as “reperfusion injury”.

In clinic, the use of preconditioning recognized as a powerful means of protection against myocardial necrosis and apoptosis, consequences of I/R, was not translational because of its intervention before ischemia. For this reason, protecting the ischemic myocardium at the reperfusion has become a necessary approach to limit the deleterious effects of I/R. For this reason, we have undertaken to review the known methods performed during reperfusion, which can be cardioprotective against damage caused by I/R. These methods can eventually be used easily in a clinical setting.

Controlled reperfusion

During ischemia-reperfusion, part of the cardiac alterations occur during reperfusion. For this reason, several studies have tried to modify the conditions of reperfusion to protect the myocardium against damage caused by I/R [41, 42]. These changes concern both the myocardium pressure of reperfusion as well as the concentration of oxygen in the physiological solution used.

The idea of changing the conditions of reperfusion to protect the ischemic myocardium started three decades ago [43]. Since that moment, several studies have been performed on different models [7, 44], and in both normothermic ischemia [7, 45] as well as hypothermic conservation [46]. However, the pathophysiological mechanisms underlying this protection were only partially identified.

Protective effect of controlled reperfusion against ischemia/reperfusion injury

Several studies have tried to change some parameters of the reperfusion to protect the ischemic myocardium against I/R injury [47, 48]. These parameters include both physical parameters (coronary flow and perfusion pressure) and chemical parameters (oxygen partial pressure, CO2, concentration of certain salts, and addition of some subtractive). Okamoto et al. tested the hypothesis that more muscle salvage after acute ischemia is possible by "gentle," temporary reperfusion than with sudden, complete revascularization [49]. Using dog model that underwent left anterior descending coronary artery ligation followed by reperfusion, they found that early temporary gentle reperfusion limits the post-ischemic damage that occurs with sudden, complete revascularization. This observation was confirmed by Peng et al. in pig model indicating that controlled reperfusion lessens end-diastolic wall thickness, reduces myocardial calcium deposition, increases the rate of mitochondrial oxidative phosphorylation, and preserves cellular high-energy phosphate stores in the ischemic-reperfused myocardium when compared to the uncontrolled reperfusion state [50]. Similar observation was also reported by Mrak et al. also in pig model [51].

Hori et al reported that an intracoronary infusion of hydrogen chloride, which mimicked the change in pH in coronary venous blood of the...
staged reperfusion, attenuated myocardial stunning [52]. In a study comparing reperfusion at pre-ischemic flow rate (9.0 ml.g-1.min-1; ordinary flow rate) and reduced flow rates (0.9-8.1 ml.g-1.min-1), Takeo et al. found that reduced flow rate reperfusion attenuated ischemia/reperfusion-induced increase in left ventricular end-diastolic pressure, alteration in tissue of Na+, K+, Ca2+, and Mg2+, release of creatine kinase and ATP metabolites [53]. Thus, in isolated guinea pig hearts, Massoudy et al. observed that controlled oxygen delivery during post-ischemic reperfusion by both, reduction of coronary flow and PO2, improves recovery of pump function and is accompanied by less oxidative stress [54]. Further, Kaneda et al. reported that high PO2 leads to myocardial reperfusion damage; however, maintaining a more physiologic PO2 during reperfusion following ischemia may attenuate reperfusion injury [55].

Recent trials from our team in isolated rat heart confirmed the protective effect of controlled reperfusion after both warm ischemia [7] and hypothermic conservation [56]. The use of controlled reperfusion has also been successfully used after hypothermic preservation of rabbit lungs [57], and has been found to exert neuroprotective effects on the spinal cord against I/R injury [58]. In a clinic setting, controlled reperfusion has been used in transplant of cardiac grafts subjected to a prolonged cold ischemia [59].

Mechanism of controlled reperfusion

Although the use of controlled reperfusion to protect the myocardium against I/R injury is well accepted, the mechanism of this protective effect still needs to be clarified and further studies are necessary to completely understand its mechanism. Takeo et al. attributed this protective effect to a limitation of the cytosolic accumulation of Na+ and Ca2+ after 35 min of global ischemia in the isolated rat heart [60]. Hori at al. demonstrated that staged reperfusion attenuates myocardial stunning via a delayed correction of acidosis during the first minutes of reperfusion [61]. Recently, in two separate studies, our group has shown that low-pressure of reperfusion induced the inhibition of the mitochondrial permeability transition pore (mPTP) opening using an isolated rat heart model after both normothermic [7] and hypothermia [56, 62] conditions, this resulted in increase of mitochondrial calcium overload and reduction of reactive oxygen species production, since low pressure is associated with a decrease of myocardial malondialdehyde [45]. The mechanism leading to inhibition of mPTP opening in controlled reperfusion-induced cardioprotection is not completely understood. However, we found that the low pressure-induced cardioprotection effect involving the up-regulation of Akt phosphorylation and inhibition of mPTP opening after I/R was prevented by the addition of both PI-3K inhibitors, wortmannin and LY294002 [63]. This work indicated that the inhibition of the mPTP opening by low-pressure of reperfusion is mediated by activation of the pro-survival PI/3K/Akt pathway. However, clarifying the precise link between PI-3K activation and inhibition of mPTP opening in low-pressure reperfusion action still needs further investigation. Thus, identifying the receptor by which the low-pressure of reperfusion acts upon will completely clarify the mechanism of this protective method.

Ischemic postconditioning

In 1986, Murry et al. showed that short cycles of I/R performed before a long and deleterious ischemia confers a cardioprotective effect against I/R injury [64]. This method to reduce the myocardial infarction was name “ischemic preconditioning”. Although this method is powerful and is easily performed in experimental conditions, its transfer in clinic setting is almost impossible. Recently, Zhao et al. had the ingenious idea of performing the very short sequences of ischemia reperfusion at the onset of the reperfusion [65]. Surprisingly, they found that this maneuver was able to reduce the infarct size comparable to that induced by preconditioning. This method was named postconditioning by opposition of preconditioning which is performed before ischemia

Protective effect of ischemic postconditioning

This discovery of Vitten-Johansen team provided clear evidence of ability to protect the ischemic myocardium at the reperfusion [66]. This approach was soon replicated by many independent teams on different models, in vivo [67, 68], ex vivo [63] and in vitro [69, 70]. The postconditioning was found to reduce the infarct size by nearly 60% after I/R [71]. Our group has shown that in isolated perfused rat heart, ischemic postconditioning decreases irreversible injury measured by lactate dehydro-
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genase, creatine kinase and troponin I release. Dosenko et al. observed in cardiomyocytes that postconditioning induces an anti-apoptotic and anti-autophagic effect after ischemia [72]. Many other effects of postconditioning were also reported like anti-arrhythmias [73], attenuation of the overproduction of ROS, and I/R-induced inflammation [74], reduction of oxidative senescence mouse [75]. The beneficial effect of postconditioning was also found in divers organs, including the brain [76, 77], liver [78, 79], kidney [80], lung [81, 82] and in intestinal mucosa barrier function [83].

The remote postconditioning effect was also reported first by Kerendi et al [84]. In fact, this group indicated that remote renal postconditioning applied immediately before the onset of coronary artery reperfusion provides significant myocardial infarct size reduction likely exerted during the first minutes of coronary artery reperfusion [85]. Remote postconditioning beneficial effects have been confirmed by many others authors [86, 87].

In clinic, Ovize team first pointed the veracity of postconditioning in helping patients with coronary disease [88]. In fact, the study of Staat et al. have shown a decrease of creatine kinase in the group of patients having a postconditioning maneuver during angioplasty compared to those having only angioplasty [89]. Further, Dragoni at al. found a significant decrease of endothelium dysfunction with postconditioning [90]. Ma et al. have confirmed this observation with patients having their first acute myocardial infarction who underwent revascularization [91]. However, these results were recently challenged by Freixa et al [92]. In fact, Freixa et al in a randomized study found that postconditioning during primary percutaneous coronary intervention (PCI) did not reduce infarct size or improve myocardial function recovery at both short- and long-term follow-up [93]. This controversy has the merit to indicate that there is still much to do in the long steps leading to the use of postconditioning in clinic setting and explained the requirement of some optimal experimental conditions when performing postconditioning.

**Experimental limits of ischemic postconditioning**

Ischemic postconditioning consists of performing brief sequences of I/R at the onset of the reperfusion. The number and the duration of cycles of I/R are the major factors to be considered for the success of postconditioning.

Kin et al. showed in rat model that postconditioning with 3 cycles of I/R of 10 seconds has the similar efficiency than with 6 cycles of I/R at the same time [67]. However, the same team reported that in mouse model that postconditioning performed with 3 sequences of I/R of 10 seconds had no effect; in contrast they observed a improvement of post-ischemic systolic and diastolic function of hearts effect when using 6 cycles of the same duration [94]. Further, Yang et al. indicated that in rabbit model that postconditioning with 4 sequences of I/R of 30 seconds has the similar efficiency than with 6 cycles of I/R [95]. Further, Staat et al observed a beneficial effect of postconditioning with 4 cycles of I/R of 60 seconds in clinic [96].

Piper et al. indicated that the first minute of the reperfusion was the critical phase for cardiomyocytes survival [97]. Thus, Kin at al showed that after the first minute of reperfusion, the postconditioning was no longer expressed [67]. In agreement with this observation, Yang al. showed in rabbit model that 10 minutes after reperfusion, that postconditioning was no longer expressed [98]. These observations were recently challenged by Roubille et al [99]. In fact, these authors have shown that in mouse model, delaying the intervention of postconditioning to 30 minutes does not abrogate the cardioprotective effect of postconditioning.

**Mechanism of postconditioning**

Although its mechanism is not completely elucidated, several studies have tried to clarify the mechanism of postconditioning. The initial report indicated that the pre-or postconditioning seemed to use the same signaling pathway [100]. An interesting hypothesis has been described by Tsang et al [101]. In this theory, the postconditioning has two modes of action: one passive and the other active (Figure 3).

The passive pathway is caused by the gradual reperfusion of postconditioning. This stress causes a decrease in the production of free radicals, a decrease of the release of neutrophils involved in inflammation, and also the decrease in mitochondrial calcium concentration.

The active pathway is represented by RISK (Reperfusion injury Salvage Kinase) that in-
volves PI-3K and induces the activation of the Akt by phosphorylation, which is the principal regulator in cell survival. The involvement of the up-regulation of Akt phosphorylation in postconditioning was confirmed by our group in isolated perfused rat heart [63]. In this study, we showed that PI-3K/Akt pathway mediates postconditioning-induced inhibition of mPTP opening. The role of the inhibition of the mPTP in postconditioning has been first showed by Argaud et al [102]. Furthermore, in a study investigating the effect of ischemic postconditioning on I/R injury in isolated hypertrophied rat heart, Pend et al. indicated that postconditioning attenuated I/R injury effect in isolated hypertrophied rat heart, which were mediated through PI-3K/Akt/GSK-3beta signaling pathway [103]. In the other hand, the RISK pathway induces activation of MAPK, involving the phosphorylation of ERK. The involvement of ERK pathway activation in postconditioning has been confirmed by the work of Darling et al. [104]. The activation of ERK and Akt leads to the translation of proteins via the phosphorylation of p70S6K protein. However, some authors have observed that postconditioning induces activation of Akt and ERK without providing a protective effect against I/R injury in the pig model [105].

Since, several studies have been performed and have helped to better understand the mechanisms of postconditioning indicated that adenosine receptors (A2A and A3) were involved in postconditioning-induced cardioprotection [67]. Serviddio et al have shown that postconditioning induces protection against infarction is associated with the decrease in the production of peroxide and reduction the glutathione [106].

In isolated ischemic rat heart, Inserte et al. showed that postconditioning delay intracellular pH recovery and prevent calpain activation, inducing cardioprotective effect against I/R [107]. However, the mechanisms by which delaying the restoration of physiological pH induces protection effect needs to still be understood [108], but it could be associated with signaling pathways activated by postconditioning [109, 110].

Although, several studies have determined signaling molecules involved in postconditioning, further studies are necessary to elucidate the mechanism of this method.

**Pharmacological postconditioning**

Besides ischemic postconditioning, which consists to perform short cycles of I/R at the onset of the reperfusion, several authors tried to protect the ischemic myocardium against I/R injury by addition of pharmacological agents in the perfusat during the reperfusion [111-113]. This method is called pharmacological postconditioning.

The team of Yellon has observed a cardioprotective effect of GIK (glucose-insulin-potassium) cocktail given during the reperfusion. The author authors also found that the protective effect induced by addition of GIK was mediated by the activation of the PI-3K/Akt pathway. The beneficial effect of GIK was later confirmed by Jonassen et al [114]. However, the benefic effect of this cocktail in clinical setting has been recently challenged [115]. In this international study named CREATE-ECLA, Randomized controlled trial conducted in 470 centers worldwide among 20,201 patients with a ST-segment elevation myocardial infarction (STEMI), the au-
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Authors conclude that high-dose GIK infusion had a neutral effect on mortality, cardiac arrest, and cardiogenic shock in patients. It is important to indicate that several independent groups have had many concerns about the clinical features of the CREATE-ECLA trial [116, 117]. To further elucidate the beneficial role of the GIK in clinic, actually an important trial named IMMEDIATE is ongoing. The result of this trial will determine the future of the use of this cocktail in clinic. Adenosine was also abundantly reported to have cardioprotective effect in pre-clinical studies [118, 119]. However, there is not unanimity in its positive effect in clinical trial AMISTAD and AMISTAD 2 [120, 121]. As reported in a recent review from Kloner and Gerczuk [122], during the last decade, several clinical trials using diverse agents, which have been reported to induce cardioprotective effect in animal model, failed to reduce injury in clinical setting (HALTMI (LeukoArrest, a CD11/CD18 leukocyte integrin receptor inhibitor), ESCAMI (Eniporide, a Na+/H+ exchange inhibitor), CASTEMI (Caldaret, an intracellular Ca2+-handling modulator), J-WIND-KATP (Nicorandil, a K+ channel opener/vasodilator), PROTECTION-AMI (Delcaseritib, a δ-protein kinase C inhibitor), REVEAL, using Erythropoietin). Anesthetic agents have been also abundantly used with success during reperfusion to protect the myocardium against damage related to I/R [123, 124]. Several studies have successfully used different pharmacological agents during the reperfusion to protect the ischemic myocardium [125-128].

Conclusion

Several pre-clinical studies have been performed in diver’s model to protect the myocardium against I/R injury. These trials indicate that it is possible to protect the ischemic myocardium during reperfusion. Controlled reperfusion and ischemic postconditioning methods were found to be able to reduce the myocardial infarct size and improve the cardiac functional recovery after both normothermic and hypothermic ischemia. The mechanism underlying the myocardial protection during reperfusion still needs further investigations. Although clinical trials using pharmacological agents has shown in many cases negative results, we have several reasons to hope that the protection of the myocardium during reperfusion by controlled reperfusion and postconditioning will be used in clinic settings.

Acknowledgements

This work was supported by the American Heart Association Fellowships 09POST2190008 (Jean Chrisostome Bopassa).

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