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High cholesterol diet effects on ischemia/reperfusion injury of the heart

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Abstract

Ischemic heart disease is the leading cause of morbi-mortality in developed countries. Both ischemia/reperfusion injury and mechanisms of cardioprotection have been studied for more than 50 years. It is known that the physiopathological mechanism of myocardial ischemia involves several factors that are closely related to its development, hypercholesterolemia which is one of the main ones. Therefore, the objective of this revision was to elucidate the effects of a high-cholesterol diet on normal ventricular function and ischemia/reperfusion injury-associated phenomenon such as post-ischemic ventricular dysfunction (stunned myocardium). Although there exist many studies considering several aspects of this physiopathological entity, the majority was carried out on normal animals.

Thus, experiments carried out on hypercholesterolemic models are controversial, in particular those evaluating different mechanisms of cardioprotection such as ischemic preconditioning and postconditioning, and cardioprotection granted by drugs such as statins which apart from exerting a lipid-lowering effect, they exert pleiotropic effects providing cardioprotection against ischemia/reperfusion injury. These controversial results regarding mechanisms of cardioprotection, vary according to: quality, composition and high-cholesterol diet time of administration as well as the different species used in each experiment.

Thus, in order to compare the results, it is necessary to take all these variables into account since they can change the obtained results.

Overview

Ischemic heart disease is the leading cause of morbi-mortality in the Western world and by 2020 it will be the leading cause of death worldwide (Roger V et al. 2011). Reperfusion therapy is the most effective treatment for ischemic disease as it has successfully reduced mortality caused by acute myocardial infarction. However, despite the fact that reperfusion therapy is nowadays considered the best therapeutic strategy to avert necrosis in the myocardium, it paradoxically produces another type of cell injury under special circumstances known as “reperfusion injury” (Jennings R et al. 1995).

It is known that the ischemic disease development is largely attributed to risk factors that can be modified such as hypercholesterolemia, which is one of the main ones. Epidemiological studies arose in the 1950s and the pioneer was the Framingham heart study (Dawber TR et al. 1951), this study showed that besides age, sex and smoking, diabetes and hypercholesterolemia constitute the major risk factors for developing CHD (coronary heart disease).

After Framingham, several studies arose and evaluated various cardiovascular risk factors, in particular hypercholesterolemia (Kannel W et al. 1966; Keys A et al. 1966; Ciruzzi M et al. 1997). Thus, the Seven Countries study showed the association between the incidence of coronary heart disease and hypercholesterolemia (Keys A et al. 1966) stating that 35.4 % of patients with acute myocardial infarction had a medical history of hypercholesterolemia while 15.5% of patients with no evidence of previous CHD events, had elevated serum cholesterol.

Although epidemiological studies have contributed enormously to determine risk factors associated with CHD, the effects of dietary cholesterol regarding the development of atherosclerosis were already known. Thus, Anitschkow and Chalatow, in 1913, for the

first time showed that high-cholesterol-fed rabbits developed a marked hypercholesterolemia and that this elevation of serum cholesterol favors the development of atherosclerosis (Anitschokow and Chalatow, 1913). Examining the importance of this risk factor, it is interesting to analyze ischemia/reperfusion injury behavior and myocardial cardioprotection mechanisms when a comorbidity is present such as hypercholesterolemia. Regarding the development of hypercholesterolemia, there are several ways in which plasma cholesterol homeostasis is regulated: a) Acetate is converted into 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG CoA) which is reduced by 3-hidroxy-3-methyl-glutaryl-coenzyme A reductase to produce mevalonic acid b) an increased expression of liver LDL receptors lowers plasma cholesterol and LDL levels; c) Dietary cholesterol; d) conversion of cholesterol into bile acid through a catabolic pathway (regulated by the enzyme cholesterol 7 alpha-hydroxylase) to excrete cholesterol. Thus, cholesterol can be endogenous and exogenous (dietary cholesterol). In clinical practice, exogenous cholesterol is interesting since modifying eating habits can help lower cholesterol leveles. Some research was carried out on laboratory animals to evaluate hypercholesterolemia as follows: a) on genetically modified animals (endogenous) (Song G. et al. 2011, Dworschak M et al. 2005); b) on high-cholesterol-fed animals (exogenous) (Bulgarelli A et al. 2011; Hadi NR et al. 2012); c) combining both (endogenous and exogenous) (Ishibashi S et al. 2011, Scalia R et al. 2001).Thus, hypercholesterolemia can be produced by endogenous or exogenous pathways.

According to the abovementioned experimental models (exogenous and endogenous), developing a hypercholesterolemic model (associated or not to an atherosclerotic disease) through a high-cholesterol diet allows us to apply conclusions in clinical practice since it represents a problem to the great majority of hypercholesterolemic

patients. Thus, this revision is focused on the study of hypercholesterolemic models to understand and compare different results.

High cholesterol diet effects on normal ventricular function

Several studies have evaluated high-cholesterol diet effects on ventricular function in normal hearts. Alterations in ventricular function were shown for the first time by Peterson et al (Peterson DW et al. 1980), these authors showed a decreased myocardial contractility in papillary muscles from rabbits which were subjected to an atherosclerotic diet (5% lard, 5% peanut oil, 0.5% cholesterol, and 89.5% rabbit pellets) for a period of time (116-184 days). They concluded that this diet produces changes in the lipid composition of the sarcoplasmic reticulum or sarcolemma or both, probably causing mechanical alterations. On the other hand, Shkliar et al (Shkliar TF et al. 1991), demonstrated that several myocardial contractility parameters decreased in a papillary muscle from atherosclerotic rats, and that these changes were probably related to a hypertrophy in the papillary muscle induced by a high-cholesterol diet. Shah et al (Shah KR et al. 1990) showed that cholesterol intake produces a decrease in contractile force, and that this alteration was independent of a vascular disease, making clear that a high-cholesterol diet may lead to a cardiac dysfunction independently of the development of atherosclerosis. These data were corroborated by Rubinstein et al (Rubinstein J et al 2009) who described myocardial alterations in high-cholesterol-fed rabbits (cholesterol 1% during 6 months), assessed by echocardiogram. These authors suggested that cholesterol deposits in the myocardium may cause a cardiomyopathy, in a similar way in which other infiltrating diseases do. Hence, myocardial metabolism may shift ATP production from glucose to free fatty acids. Thus, Krebs cycle increases free radicals leading to a

myocardial injury. In agreement with these findings, we have detected in a previous study a negative inotropic effect of hypercholesterolemia on ventricular function in high-cholesterol-fed rabbits (cholesterol 1% during 4 weeks) (D´Annunzio V et al. 2005). In this study we have shown a decreased of 22.2±3.9% in left ventricular developed pressure on high-cholesterol-fed animals (figure 1, panel A). In contrast to other studies, we have detected these changes on isolated rabbit hearts which were then perfused according to Langendorff technique allowing us to keep the variables that modulate the ventricular function constant and under control. Regarding diastolic function, in contrast to other authors, we did not detect the diastolic relaxation altered but an increase in myocardial stiffness (D´Annunzio V et al. 2005) (figure 1, panel C and D). However, contrasting our study, Huang et al. (Huang Y et al. 2004) found in a model of isolated myocytes, that a high-cholesterol diet during 10 weeks produced an early decrease in SERCA's ARNm levels (at the 8-day diet period), however, alterations in relaxation and contractility were produced at the end of the 10-week diet period. Thus, they concluded that a high-cholesterol diet induces a kind of cardiomyopathy, characterized by systolic and diastolic ventricular dysfunction. In agreement, Zhai et al. (Zhai Y et al. 2008) showed that high-cholesterol-fed pigs (cholesterol 2% during 12 weeks) evidenced an increased myocardial fibrosis, through the activation of TGF-β and that this activation produced diastolic ventricular dysfunction. All these changes were reversed when antioxidants (vitamin E y C) were supplemented to the high-cholesterol diet. In this way, they concluded that the high-cholesterol diet produces early changes in diastolic ventricular function, due to a TGF-β rise which causes an increase of the oxidative stress. In contrast, our study suggests that differences of the diastolic component could be related to differences in time of administration and concentration of the diet. In other studies, where basal diastolic parameters were altered through a high-cholesterol diet, functional changes were evident

in animals subjected to a diet for longer periods of time (6 months, 10 and 12 weeks). Our results were different as we have only administered the diet for 4 weeks. Thus, it is clear that diastolic changes are detected later than systolic ones that are detected earlier. Talini et al (Talini E et al. 2008) demonstrated similar findings in hypercholesterolemic patients with no evidence of coronary artery disease, although, they presented subclinical myocardial abnormalities regarding global systolic and diastolic function that were detected by tissue doppler and myocardial performance index. These alterations were reversed after 6 months of treatment with rosuvastatin.

Although there is enough experimental evidence demonstrating that cholesterol modulates ventricular function, Toleikis et al. (Toleikis PM and Tomlinson CW 1987) did not detect a ventricular dysfunction when applying a high-cholesterol diet (cholesterol 2% during 8 weeks), which doubles concentration and diet time of administration applied in our study. Thus, these authors have probably performed the abovementioned study in presence of atherosclerotic injuries. In agreement, Tilton et al (Tilton RG et al. 1987) has neither detected basal functional changes in high-cholesterol- fed rabbits (cholesterol 2%) during 2-3 weeks, but it has in the sixteenth week, thus 2-3 weeks were probably not enough to develop ventricular diastolic alteration. In a similar way, Bauersachs et al. (Bauersachs J et al. 2006), showed that hypercholesterolemia itself did not affect LV function in sham-operated animals but in high-cholesterol-fed rabbits (0.25% cholesterol during 4 weeks) with myocardial infarction, the impaired left ventricular systolic and diastolic function got worse comparing it to normocholesterolmic animals.

Thus, based on experimental evidence, we can suggest that a low-cholesterol diet (0.25%, in Bauersachs J et al. 2006) or a short-term diet (2/3 weeks, Tilton RG et al. 1987) could not be enough to develop functional alterations and, that at least 4 weeks and ≥ 1%

concentrations, could be necessary to detect ventricular function alterations. Therefore, it is clear that a high-cholesterol diet causes changes in the ventricular function behavior, however, after detailed analysis of the different functional parameters (contractility, relaxation and myocardial stiffness), not all behave in the same way, and the presence or absence of changes in functional parameters could depend on several variables. Thus, it is possible to speculate that systolic alterations occur in earlier phases of hypercholesterolemia, while alterations in the diastolic function occur in models which have been subjected to a diet for a longer period of time (≥ 10 weeks).

In summary, alterations in functional parameters principally depend on time and diet concentration applied to the different experimental models. These may be caused by an alteration in the myocardial metabolism and/or by changes in the composition of the sarcolemmal and sarcoplasmic reticulum membranes (figure 2).

High- cholesterol diet effects on myocardial stunning

To the best of our knowledge, only a few studies have evaluated ventricular function independently of the study of the infarct area in hypercholesterolemic animals, in particular the stunned myocardium phenomenon. Regarding these, Le Grand et al. (Le Grand B et al. 1995) demonstrated that high-cholesterol-fed rabbits paradoxically had a major resistance against injury produced by a 30 min of ischemia. On the other hand, Van de Velde et al. (Van de Velde M et al. 2000) demonstrated that a triglyceride emulsion administered during reperfusion enhances the recovery of the function and the metabolic state of the stunned myocardium in isolated rabbits' hearts. Satoh et al (Satoh K et al. 2008), showed in a dog model with stunned myocardium and normal diet, that pitavastatin reduced plasma cholesterol levels (25%) and post-ischemic contractile dysfunction, in the

absence of a high-cholesterol diet. In agreement with several authors, we have published that high-cholesterol fed animals' hearts (1% cholesterol during 4 weeks) subjected to an ischemia protocol (15 min) and reperfusion (30 min), evidenced a significant recovery of the contractile state, as well as a reduced myocardial stiffness compared to the normocholesterolemic animals, with no changes in the isovolumic relaxation (figure 1) (D´Annunzio V et al. 2005). It is important to highlight that when isoproterenol was administered to match the preischemic inotropic state in normocholesterolemic animals' hearts, the protective effect on post-ischemic ventricular dysfunction was abolished. Our findings suggest that low inotropic state of hypercholesterolemic rabbits' hearts could reduce myocardial oxygen consumption to protect the myocardium. It is noteworthy that our results reflect a beneficial effect of the high-cholesterol diet on ventricular function since infarct size was not highly significant during 15 min of ischemia, thus, this variable was no longer necessary (D´Annunzio V et al. 2005). It is important to highlight, as it was previously mentioned, that several authors have detected a lower contractile state on hypercholesterolemic models since cholesterol interferes in the membranes' permeability and channels (Huang Y et al 2004, Peterson DW. et al 1980). In agreement with this statement, Luo et al (Luo TY et al. 2004), demonstrated that high-cholesterol-fed rabbits (cholesterol 0.5% and coconut oil during 12 weeks) evidenced a decrease in the contractility produced by a minor expression in the SERCA channel and an increase in the Na^{+}/Ca^{++} exchanger expression. These changes detected in the channels, which are involved in contractility, could explain a lower inotropism in hypercholesterolemic animals. Thus, a decreased inotropic state before ischemia could be the reason why the heart tolerates ischemia in a better way.

On the other hand, both Van de Velde (Van de Velde M et al.2000) and Calabresi et al. (Calabresi L et al. 2002), who administered an HDL cholesterol infusion on isolated

and perfused rat hearts, found cardioprotection in a stunned-heart model as we did. However, they acutely administered emulsions during the experiment, which differs from our model where ventricular function behavior was evaluated in a 4-week-high-cholesterol diet. Regarding our findings, Kalaivanisailaja et al. (Kalaivanisailaja J et al.2003) demonstrated that a high-cholesterol diet produces an increase of free fatty acids, and, as it has been shown by Van de Velde et al. (Van de Velde M et al.2000) fatty acids have a protective effect against ischemic/reperfusion injury. Thus, in our experimental model, this mechanism could also be part of the cardioprotection.

In summary, experimental evidence suggest that hypercholesterolemia in an early phase, could produce a decrease in the contractile state, which is an oxygen consumption determinant, and, in this way, a reduction of inotropism induced by hypercholesterolemia could at least explain the possible protective mechanism (figure 2). These changes occur by alterations in the permeability of the membranes and channels involved in contraction. Another possible explanation is that a plasma cholesterol increase can produce a rise of free fatty acids and these could produce a direct protective effect against post-ischemic ventricular dysfunction (stunned myocardium). It is important to highlight that hypercholesterolemia could involve in this way the mechanisms abovementioned, and thus, the ventricular dysfunction is recovered after a brief episode of ischemia. However, more studies will be needed to clarify physiopathological mechanisms involved in the cardioprotection against stunned myocardium.

High-cholesterol diet effects on infarct size

Regarding variations in infarct size and high-cholesterol diet, in 1987 Golino et al. (Golino P et al.1987), showed for the first time that hypercholesterolemia per se produced a significant increase of infarct size compared to normal fed animals. They showed that a

high-cholesterol diet (cholesterol 2%) during 3 days was sufficient time to induce moderate hypercholesterolemia (about 330 mg/dl as compared to a control value of 67 mg/dl), but was not sufficient to produce any detectable atherosclerotic vascular changes. The most relevant results of this study showed that infarct size was doubled after coronary artery occlusion-reperfusion in hypercholesterolemic rabbits. In the same way, Wang et al. (Wang TD et al.2002) showed that cholesterol administration produced an increase in both infarct size and quantity of apoptotic cells in the ischemic area, and that this larger infarct size was related to the capase 1 activity. In a similar way, Sakamoto et al. (Sakamoto S et al.1991) observed in awake dogs that infarct size was larger in high-cholesterol-fed dogs. Recently, Szucs et al (Szucs G et al. 2011) have shown that a high-cholesterol diet (2% cholesterol during 9 weeks) increases infarct size after 30 min of global ischemia and 120 min of reperfusion. This infarct size increase could be related to a rise of the metalloproteinase type 2 (MMP-2) activity during reperfusion regarding normal fed animals, reflecting on an indirect way an oxidative stress rise on hypercholesterolemic animals since it has already been shown that an increase of the oxidative stress, through the peroxynitrite formation, activates MMP-2 (Wang TD et al. 2002). Thus, this enzyme could participate in the reperfusion/ischemia injury (Szucs G et al. 2011). Osipov et al (Osipov RM et al. 2009) subjected pigs to a high-fat/high-cholesterol diet (Sinclair Research Center, Inc) during 20 weeks to demonstrate that hypercholesterolemia is associated with myocardial oxidative stress increase, inflammation, reduction of cell survival pathways and induction of apoptosis in the ischemic territory, which together may account for the expansion of myocardial necrosis in the setting of acute ischemia/reperfusion. These findings are in agreement with our previously published results, since we have also showed that infarct size on high-cholesterol-fed rabbits (cholesterol 1% during 4 weeks) increased significantly after 30 min of global ischemia and

120 min of reperfusion (figure 3, panel A). Although in our experimental model we did not detect atheromatous plaques neither in coronary arteries nor in intramyocardial vessels, we found that by increasing the dose of acetylcholine infusion, a vasoconstrictor effect was produced, interpreting it as an alteration in the vasodilation response handled by the endothelium (Donato M et al.2007).

As a conclusion, it is clear that every model where a hypercholesterolemia is developed, in presence or absence of atherosclerosis, brings about an increase of the oxidative stress and that this worsens ischemia/reperfusion injury, increasing infarct size (Donato M et al. 2007; Sucz G et al 2011; Van Craeyveld E et al. 2011; Iliodromitis EK et al. 2010).

High-cholesterol diet effects on myocardial protection mechanisms.

Ischemic preconditioning and posconditioning

Reviewing experiments that have studied ischemic preconditioning and posconditioning effects in hypercholesterolemic animals is interesting since results are contradictory (Szilvassy Z et al.1997; Ferdinandy P et al.1997; Ueda Y et al.1999). A pioneer study by Szilvassy al (Szilvassy Z et al.1997) in which preconditioning effects in hypercholesterolemic animals were evaluated, it was demonstrated that preconditioning cardioprotection is abolished in hypercholesterolemic and atherosclerotic rabbits (1.5% high-cholesterol diet during 8 weeks). When these animals switched from a high-cholesterol diet to a normal one and serum lipid levels reached similar values to basal ones, the preconditioning protective effect was again induced, even in the presence of atherosclerosis. These results indicate that hypercholesterolemia, independently of

atherosclerosis development, interferes with preconditioning cardioprotection mechanisms. In agreement, Ferdinandy et al. (Ferdinandy P et al.1997), showed no reduction of the infarct size on preconditioned rats which were fed with a high-cholesterol diet (cholesterol 2% during 12 weeks), attributing this to an alteration of nitric oxide biodisponibility. The same investigation board in a further study, demonstrated in high-cholesterol-fed rats (cholesterol 2% during 12 weeks) that preconditioning cardioprotective effect was lost due to a superoxide anion and conexine 43 increase on hypercholesterolemic animals (Görbe A et al. 2011). In a similar way, Ueda et al. (Ueda Y et al.1999) published similar results; and Tang et al (Tang et al. 2004) showed that a larger amount of preconditioning cycles could increase infarct size on isolated rabbits' hearts which were subjected to ischemia/reperfusion after an 8-week-high-cholesterol diet. Recently, Kocsis et al (Kocsis G et al. 2010) concluded that a high-cholesterol diet (cholesterol 2% during 8 weeks) leads to alterations in preconditioning-induced gene expression in the mouse heart, which might lead to marked changes of oxidative/nitrosative stress signaling and to reducing the preconditioning cardioprotective. Loss of cardioprotection was also detected in patients (Kyriakides ZS et al.2001; Juhasz B et al.2004). Thus, Kyriakides et al (Kyriakides ZS et al.2001), described protective effect loss (evaluated through ST-segment elevation) in patients who underwent a coronary angioplasty. There is a positive correlation between plasma levels of LDL and preconditioning cardioprotection loss.

Although there exist experimental evidence suggesting that preconditioning protective effect is lost during hypercholesterolemia, there are several studies contrasting those showing that the cardioprotective effect persists even when plasma cholesterol levels are high. Thus, we showed that preconditioning significantly decreased infarct size with no significant recovery of ventricular function (Figure 3 panel B) in an isolated rabbit heart (1% high-cholesterol diet during 4 weeks). The reduction percentage of infarct size

was larger on hypercholesterolemic animals if we compare it to normal ones. Regarding to our findings, Kremastinos et al. (Kremastinos DT et al.2000), using a high-cholesterol-fed rabbit model (cholesterol 2% and maize oil 6% during 8 weeks), showed that preconditioning reduces infarct size as in normal animals. Similar findings were described by other authors such as Iliodromitis et al. (Iliodromitis EK et al.2006), who applied a high-cholesterol diet for 6 weeks, and by Jung et al. (Jung O et al.2000) with a 4-week diet. In a similar way, Dworakowski et al. (Dworakowski R et al. 2008) in a rat model of papillary muscle demonstrated that a high-cholesterol diet did not change preconditioning beneficial effects in a guinea pig model subjected to a high-cholesterol diet for 5 weeks.

In summary, and considering several authors studies, we can conclude that animals who were fed with cholesterol during short periods of time (4-5 weeks), preconditioning was effective, while animals which were fed for longer periods of time (≥8 weeks), abolished the preconditioning protective effect with the exception of Kremastinos et al study (Kremastinos DT et al 2000), in which an 8-week-high-cholesterol diet did not abolish the preconditioning protective effect. However, this study was carried out on rabbits with severe atherosclerotic lesions. Having all these experimental evidence, we can conclude that it is probable that a high-cholesterol diet produces intracellular changes which interfere with intracellular mechanisms involved in cardioprotection granted by ischemic preconditioning.

Regarding ischemic postconditioning, there exist few studies evaluating postconditioning effects on hypercholesterolemic animals. Thus, Iliodromitis et al. (Iliodromitis EK et al.2006) showed that postconditioning did not reduce infarct size on hypercholesterolemic rabbits suffering from an advanced phase of atherosclerosis, since there were numerous subintimal lipid deposits in the coronary arteries with a significant

reduction of the artery lumen. In agreement, Kupai et al., (Kupai K et al. 2009) using a high-cholesterol diet (cholesterol 2% during 12 weeks), could not show postconditioning protective effects (6 cycles 10 seconds) on isolated rat hearts. These authors showed that an early increase in peroxynitrite-induced nitrosative stress after postconditioning is involved in the triggering mechanism of cardioprotection by postconditioning and that, in hyperlipidemia, the absence of this mechanism may contribute to the loss of postconditioning in hyperlipidemia. Contrasting these studies, we demonstrated that postconditioning performed in 2 cycles of reperfusion/ischemia (30 sec each) reduces infarct size on high-cholesterol-fed rabbits (cholesterol 1% during 4 weeks) (Donato M et al.2007). It is important to highlight that in our model, as it was mentioned, there are no atherosclerotic injuries in the coronary arteries but an endothelial dysfunction does exist. Differences in results (in presence or absence of the postconditioning protective effect) could lie in the fact that when the protective effect was abolished, hypercholesterolemia was associated to atherosclerosis, while the model in which the protective effect persisted, was in absence of atherosclerotic injuries. On the other hand, differences in findings could be attributed to the fact that authors used different variables such as: type of specie, diet type and duration, etc. Particularly, in postconditioning there exist another variable to be analyzed regarding quantity and postconditioning cycle's length. Illiodromitis et al (Iliodromitis EK et al. 2010) used a postconditioning protocol of 6 cycles of 10 sec each and 4 cycles of 30 sec each. Differences in cycles and type of diet could be the reason why the results are different. In our experimental model we found cardioprotection when performing 2 cycles of reperfusion/ischemia. To the best of our knowledge, there are no studies using 2 cycles, most of them have performed experiments using 3, 4 or 6 cycles. Regarding this, Vinten Johansen's research group suggested that cycle's quantity is not important but cycle's length. On the other hand, Schwartz and colet al (Schwartz L et al.

2006) showed that 3 cycles of 30 sec each performed on pigs did not achieve infarct size reduction. Based on this, Penna et al. (Penna C et al. 2008) suggested that longer cycles of ischemia/reperfusion are required to find cardioprotection, while in smaller animals shorter periods of time are sufficient. Thus, in our rabbit model, 2 cycles of 30 sec could be enough to activate postconditioning cardioprotection mechanism in both normocholesterolemic and hypercholesterolemic animals.

As a conclusion, it is clear that cardioprotection granted by cardioprotection mechanisms in hypercholesterolemic models involve several factors (type of specie, diet type and duration, presence or absence of atherosclerosis and the cardioprotection mechanism protocol used), that can support differences between the obtained results. Thus, it is necessary to be careful when comparing the obtained results in hypercholesterolemic animals.

Statins

Statins are HMGCoA reductase inhibitors and are known for their lipid-lowering effects (Mosca S et al. 2002; Ginsberg H et al. 1995; Hoeg J et al 1987), however, some effects have been attributed to them independently of their lipid-lowering effect called pleiotropic effects (Adameova A et al. 2009; Bell RM and Yellon DM 2003). During the last years, several studies demonstrated that statins protect myocardium from ischemia/reperfusion injury and that can also be beneficial for patients with acute coronary syndrome (ACS) (Di Napoli et al.2005; Ikeda et al. 2003; Jones et al. 2001; Tiefenbacher C et al. 2003 Bell RM and Yellon DM 2003; Thuc L et al. 2010). Regarding this concept, Jones et al. (Jones SP et al.2001) showed that a pre-treatment with simvastatin reduces infarct size and ventricular dysfunction after 30 min of regional ischemia and 24 hs of

reperfusion in rats. These cardioprotective effects were also detected after chronic administration of simvastatin (6 months), showing a long-term effect. However, the cardioprotection reached in this model was not detected until the drug was administered at least 3 hs before myocardial ischemia (Jones SP et al.2001). On the other hand, Tiefenbacher et al. (Tiefenbacher C et al.2003) showed that fluvastatin intravenous administration before a regional ischemia episode followed by a constant intravenous infusion during ischemia and reperfusion, reduces infarct size and enhances the recovery of the ventricular function and myocardial perfusion. However, when L-NAME (nitric oxide (NO) synthase inhibitor) was administered, the protective effect of fluvastatin was abolished, showing that statin acute administration reduces ischemia/reperfusion injury through a mechanism which involves NO. Furthermore, Čarnická et al (Čarnická et al. 2011) demonstrated that acute treatment with lipophilic simvastatin and hydrophilic pravastatin suppressed the severity of reperfusion-induced tachyarrhythmia and reduced lethal injury in rat hearts. While infarct size-limiting effect was marked in hearts treated with pravastatin, simvastatin only improved post-ischemic recovery of myocardial function (figure 4). Considering the physical-chemical and pharmacokinetic properties of statins, they may provide a basis for a rational choice of a preferable agent to avert different signs of acute myocardial ischemia including arrhythmias, myocardial infarction and contractile dysfunction. In 2003, Bell et al. (Bell RM and Yellon DM 2003) showed that atorvastatin administration during reperfusion, after 35 min of global ischemia, significantly decreases infarct size. These authors showed that in this mechanism of acute cardioprotection, phosphoinositide 3 Kinase (PI3K), Akt and NOS system were involved, all these proteins that are known for being part of the enzyme system of the RISK pathway. This earlier activation of the PI3K/Akt system rises and rapidly increases NO disponibility, molecule that is in charge of producing the protective effect. In a previously published study by our

research group, we demonstrated that acute administration of rosuvastatin during reperfusion, significantly decreased infarct size in normal and hypercholesterolemic animals. We have also found a significant improvement of post-ischemic ventricular dysfunction in high-cholesterol-fed animals which were treated with rosuvastatin during reperfusion. Other interesting finding was that MMP-2 activity was reduced during reperfusion in both groups of animals treated with rosuvastatin. This MMP-2 activity was significantly correlated with infarct size, strongly suggesting the participation of MMP-2 in ischemia/reperfusion myocardial injury (D´Annunzio et al. 2008) (figure 3). As mentioned, MMP-2 is essentially activated by the presence of peroxynitrite (Wang et al. 2002). It has been widely demonstrated that statins exert their protective effects through an increase of NO (Birnbaum et al. 2008; Tiefenbacher C et al.2003; Balakumar et al.2011; Alí et al. 2009), thus, it could be possible to hypothesize that when rosuvastatin rises biodisponibility of NO, it could reduce the peroxynitrite increase which occurs during reperfusion, and in this way, it could be capable of decreasing MMP-2 activation and, thus, reducing ischemia/reperfusion injury. However, we cannot dismiss that acute administration of rosuvastatin during reperfusion involves the activation of IP3K and Akt (kinases that participate in cardioprotection mechanisms), Bell et al. (Bell RM and Yellon DM 2003). These studies which showed that statins grant cardioprotection against ischemia/reperfusion injury, were mainly carried out on animals with normal lipid levels. To the best of our knowledge, there are little few studies that have evaluated the role of acute administration of statins comparing their effects in normocholesterolemic and hypercholesterolemic animals. Thus, in a previously published study, our group showed that the acute administration of rosuvastatin, during reperfusion, significantly decreased infarct size in normal and hypercholesterolemic animals (Figure 3, panel C). We also showed that there was a significant improvement in the post-ischemic ventricular

dysfunction in high-cholesterol-fed animals which were treated with rosuvastatin during reperfusion. Another interesting finding was the reduction of MMP-2 activity during reperfusion in both groups of animals treated with rosuvastatin. On the other hand, when doxycycline (a nonspecific inhibitor of MMPs) was administered, infarct size decreased (Figure 3 panel D). In a similar way, Adameova et al (Adameova et al. 2009) showed in a rat model subjected to ischemia/reperfusion, that simvastatin treatment (5 days before sacrificed) improved ventricular function and reduced arrhythmia in normal, hypercholesterolemic and diabetic animals (1 % cholesterol, 1 % cocounut oil; 20 g/kg per day). The present study demonstrated that the cardioprotection afforded by statins was independent of cholesterol lowering, but in this study, treatment was not acute but chronic.

As a conclusion, it is clear that statins exert a protective effect against ischemia/reperfusion injury in both normocholesterolemic and hypercholesterolemic experimental models. It is important to highlight that the administration of these lipid-lowering drugs activate mechanisms of cardioprotection such as NO, RISK pathway, decrease production of reactive oxygen species and peroxynitrites' reduction, thus, reducing MMP-2 activity. Interestingly, all these protective systems are activated after the acute administration of statins which is interesting in clinical practice.

Conclusion

Finally, we can conclude that a high-cholesterol diet, independently of the presence or absence of atherosclerosis, modules the ventricular function under baseline conditions. This occurs due to an alteration in the sarcolemmal channels and the sarcoplasmic reticulum produced by hypercholesterolemia that triggers a negative inotropism effect. This leads to a lower myocardial oxygen consumption, thus, the recovery of post-ischemic

ventricular dysfunction is enhanced (stunned myocardium) (figure 2). It is also clear that statins, independently of their lipid-lowering effects, have a beneficial effect on ischemia/reperfusion injury. On the other hand, results from physiological cardioprotection mechanisms (ischemic preconditioning and postconditioning) are controversial as the beneficial effects depend on many variables such as: type of specie, type and duration of diet and presence or absence of atherosclerosis. Hence, it is necessary to be careful when comparing results (regarding the presence or absence of ischemic preconditioning and postconditioning beneficial effects) and when applying them in clinical practice. Thus, only those studies that were performed using the same variables can be compared.

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References

Adameova A, Harcarova A, Matejikova J, Pancza D, Kuzelova M, Carnickas S, Svec P, Bartekova M, Stik J Ravingerová T 2009. Simvastatin alleviates myocardial contractile dysfunction and lethal ischemic injury in rat heart independent of cholesterol-lowering effects. Physiol Res; 58(3):449-54. PMID: 19627175

Ali F, Zakkar M, Karu K, Lidington EA, Hamdulay SS, Boyle JJ, Zloh M, Bauer A, Haskard DO, Evans PC, Mason JC 2009. J Induction of the cytoprotective enzyme heme oxygenase-1 by statins is enhanced in vascular endothelium exposed to laminar shear stress and impaired by disturbed flow. Biol Chem ; 284(28):18882-92. PMID: 19457866

Anitschokow N and Chalatow S 1983. On experimental cholesterin steatosis and its significant in the origin of some pathological procceses. Zentrahl. Allg. Pathol. 24:1-9. Transl by Z. Pelias Arteriosclerosis; 3:178-183. PMID: 6340651.

Balakumar P, Kathuria S, Taneja G, Kalra S, Mahadevan N 2011. Is targeting eNOS a key mechanistic insight of cardiovascular defensive potentials of statins? J Mol Cell Cardiol; 24 (in press). PMID: 21968328.

Bauersachs J, Hiss K, Fraccarollo D, Laufs U, Ruetten H 2006. Simvastatin improves left ventricular function after myocardial infarction in hypercholesterolemic rabbits by anti-inflammatory effects. Cardiovasc Res; 72(3):438-46. PMID: 17026973.

Bell RM, Yellon DM 2003. Atorvastatin, administered at the onset of reperfusion, and independent of lipid lowering, protects the myocardium by upregulating a pro-survival pathway. J Am Coll Cardiol; 41:508-515. PMID: 12575984.

Birnbaum Y, Lin Y, Ye Y, Merla R, Perez-Polo JR, Uretsky BF 2008. Pretreatment with high-dose statin, but not low-dose statin, ezetimibe, or the combination of low-dose statin and ezetimibe, limits infarct size in the rat. J Cardiovasc Pharmacol Ther; 13(1):72-9. PMID: 18287593.

Bulgarelli A, Caramelli B, Maranhão RC 2011. Treatment with methotrexate inhibits atherogenesis in cholesterol-fed rabbits. J Cardiovasc Pharmacol; Nov 21. Epub ahead of print. PMID: 22113347.

Calabresi L, Gomaraschi M, Villa B, Omoboni L, Dmitrieff C, Franceschini G 2002. Elevated soluble cellular adhesion molecules in subjects with low HDL-cholesterol. Arterioscler Thromb Vasc Biol; 22: 656-661. PMID: 11950706.

Čarnická S, Adameová A, Nemčeková M, Matejíková J, Pancza D, Ravingerová T 2011. Distinct effects of acute pretreatment with lipophilic and hydrophilic statins on myocardial stunning, arrhythmias and lethal injury in the rat heart subjected to ischemia/reperfusion. Physiol Res; 60(5):825-30. PMID: 22106818.

Ciruzzi M, Schargrodsky H, Rozlosnik J, Pramparo P, Delmonte H, Rudich V, Piskorz D, Negri E, Soifer S, La Vecchia C 1997. Frequency of family history of acute myocardial infarction in patients with acute myocardial infarction. Argentine FRICAS (Factores de Riesgo Coronario en America del Sur) Investigators. Am J Cardiol; 80:122-127. PMID: 9230145.

D'Annunzio V, Donato M, Erni L, Miksztowicz V, Buchholz B, Carrión CL, Schreier L, Wikinski R, Gelpi RJ, Berg G, Basso N 2009. Rosuvastatin given during reperfusion decreases infarct size and inhibits matrix metalloproteinase-2 activity in

normocholesterolemic and hypercholesterolemic rabbits. J Cardiovasc Pharmacol; 53(2):137-44. PMID: 19188835.

D'Annunzio V, Donato M, Sabán M, Sanguinetti SM, Wikinski RL, Gelpi RJ 2005. Hypercholesterolemia attenuates postischemic ventricular dysfunction in the isolated rabbit heart. Mol Cell Biochem; 273(1-2):137-43. PMID: 16013448.

Dawber TR, Meadors GF, Moore FE Jr 1951. Epidemiological approaches to heart disease: the Framingham Study. Am J Public Health Nations Health; 41:279-281. PMID: 14819398.

Di Napoli P, Taccardi AA, Grilli A, De Lutiis MA, Barsotti A, Felaco M, De Caterina R 2005. Chronic treatment with rosuvastatin modulates nitric oxide synthase expression and reduces ischemia-reperfusion injury in rat hearts. Cardiovasc Research; 66(3):427-9. PMID: 15914111.

Donato M, D'Annunzio V, Berg G, Gonzalez G, Schreier L, Morales C, Wikinski RL, Gelpi RJ 2007. Ischemic postconditioning reduces infarct size by activation of A1 receptors and K+(ATP) channels in both normal and hypercholesterolemic rabbits. J Cardiovasc Pharmacol; 49(5):287-92. PMID: 17513947.

Dworakowski R, Dworakowska D, Kocic I, Wirth T, Gruchała M, Kamiński M, Ray R, Petrusewicz J, Yla-Herttuala S, Rynkiewicz A 2008. Experimental hyperlipidaemia does not prevent preconditioning and it reduces ischemia-induced apoptosis. Int J Cardiol; 126(1):62-7. PMID: 17482295.

Dworschak M, d'Uscio LV, Breukelmann D, Hannon JD 2005. Increased tolerance to hypoxic metabolic inhibition and reoxygenation of cardiomyocytes from apolipoprotein E-deficient mice. Am J Physiol Heart Circ Physiol; 289(1):H160-7. PMID: 15734885.

Ferdinandy P, Szilvássy Z, Horváth LI, Csont T, Csonka C, Nagy E, Szentgyörgyi R, Nagy I, Koltai M, Dux L 1997. Loss of pacing-induced preconditioning in rat hearts: role of nitric oxide and cholesterol-enriched diet. J Mol Cell Cardiol; 29:3321-3333. PMID: 9441838.

Ginsberg HN 1995. Update on the treatment of hypercholesterolemia, with a focus on HMG-CoA reductase inhibitors and combination regimens. Clin Cardiol; 18(6):307-15. Review. PMID: 7664504.

Golino P, Maroko PR, Carew TE 1987. The effect of acute hypercholesterolemia on myocardial infarct size and the no-reflow phenomenon during coronary occlusion-reperfusion. Circulation; 75:292-298. PMID: 3791611.

Görbe A, Varga ZV, Kupai K, Bencsik P, Kocsis GF, Csont T, Boengler K, Schulz R, Ferdinandy P 2011. Cholesterol diet leads to attenuation of ischemic preconditioning-induced cardiac protection: the role of connexin 43. Am J Physiol Heart Circ Physiol; 300(5):H1907-13. PMID: 21398600.

Hadi NR, Al-Amran F, Hussein MA, Rezeg FA 2012. Evaluation of the effects of glimepiride (Amaryl) and repaglinide (novoNorm) on atherosclerosis progression in high cholesterol-fed male rabbits. J Cardiovasc Dis Res;3(1):5-11.PMID: 22346138

Hoeg JM, Brewer HB Jr 1987. 3-Hydroxy-3-methylglutaryl--coenzyme A reductase inhibitors in the treatment of hypercholesterolemia. JAMA; 258(24):3532-6 PMID: 3316727.

Huang Y, Walker KE, Hanley F, Narula J, Houser SR, Tulenko TN 2004. Cardiac systolic and diastolic dysfunction after a cholesterol-rich diet. Circulation; 109:197-102. PMID: 14676147.

Ikeda Y, Young LH, Lefer AM 2003. Rosuvastatin, a new HMG-CoA reductase inhibitor, protects ischemic reperfused myocardium in normocholesterolemic rats. Cardiovasc Pharmacol; 41:649-656. PMID: 12658068.

Iliodromitis EK, Andreadou I, Prokovas E, Zoga A, Farmakis D, Fotopoulou T, Ioannidis K, Paraskevaidis IA, Kremastinos DT 2010. Simvastatin in contrast to postconditioning reduces infarct size in hyperlipidemic rabbits: possible role of oxidative/nitrosative stress attenuation. Basic Res Cardiol; 105(2):193-203. PMID: 20066537.

Iliodromitis EK, Zoga A, Vrettou A, Andreadou I, Paraskevaidis IA, Kaklamanis L, Kremastinos DT 2006. The effectiveness of postconditioning and preconditioning on infarct size in hypercholesterolemic and normal anesthetized rabbits. Atherosclerosis; 188: 356-376. PMID: 16376892.

Ishibashi S, Goldstein JL, Brown MS, Herz J, Burns DK 1994. Massive xanthomatosis and atherosclerosis in cholesterol-fed low density lipoprotein receptor-negative mice. J Clin Invest; 93(5):1885-93.

Jennings R, Steenbergen C, Reimer K 1995. Myocardial Ischemia and Reperfusion. Cardiovascular Pathology International Academy of Pathology Monograph Schoen and Gimbrone Editors, Williams & Wilkins. 47-79.

Jones SP, Trocha AD, Lefer DJ 2001. Pre-treatment with simvastatin attenuates myocardial dysfunction after ischemia and chronic reperfusion. Arterioscler Thromb Vasc Biol; 21:2059-2064. PMID: 11742885.

Jung O, Jung W, Malinski T, Wiemer G, Schoelkens BA, Linz W 2000. Ischemic preconditioning and infarct mass: the effect of hypercholesterolemia and endothelial dysfunction. Clin Exp Hypertens; 22(2):165-79. PMID: 10744357.

Kalaivanisailaja J, Manju V 2003. Lipid profile in mice fed a high-fat diet after exogenous leptin administration. Pol J Pharmacol; 55:763-769. PMID: 14704473.

KanneL WB, DAwber TR, Kagan A, Revotskie N, Stokes J 1961. 3rd Factors of risk in the development of coronary heart disease--six year follow-up experience. The Framingham Study. Ann Intern Med; 55:33-50. PMID: 13751193.

Keys A, Aravanis C, Blackburn HW, Van Buchem FS, Buzina R, Djordjević BD, Dontas AS, Fidanza F, Karvonen MJ, Kimura N, Lekos D, Monti M, Puddu V, Taylor 1966. Epidemiological studies related to coronary heart disease: characteristics of men aged 40-59 in seven countries. Acta Med Scand Suppl; 460:1-392. PMID: 5226858.

Kocsis GF, Csont T, Varga-Orvos Z, Puskas LG, Murlasits Z, Ferdinandy P 2010. Expression of genes related to oxidative/nitrosative stress in mouse hearts: effect of preconditioning and cholesterol diet. Med Sci Monit;16(1):BR32-9.PMID:20037483

Kremastinos DT, Bofilis E, Karavolias GK, Papalois A, Kaklamanis L, Iliodromitis EK 2000. Preconditioning limits myocardial infarct size in hypercholesterolemic rabbits. Atherosclerosis; 150:81-89. PMID: 10781638.

Kyriakides ZS, Psychari S, Iliodromitis EK, Kolettis TM, Sbarouni E, Kremastinos DT 2002. Hyperlipidemia prevents the expected reduction of myocardial ischemia on repeated balloon inflations during angioplasty. Chest; 121:1211-1215. PMID: 11948055.

Kupai K, Csonka C, Fekete V, Odendaal L, Van Rooyen J, Marais de W, Csont T, Ferdinandy P 2009. Cholesterol diet-induced hyperlipidemia impairs the cardioprotective effect of postconditioning: role of peroxynitrite. Am J Physiol Heart Circ Physiol; 297(5):H1729-35. PMID: 19734363.

Le Grand B, Vié B, Fauré P, Degryse AD, Mouillard P, John GW 1995. Increased resistance to ischaemic injury in the isolated perfused atherosclerotic heart of the cholesterol-fed rabbit. Cardiovasc Res; 30:689-696. PMID: 8595614.

Luo TY, Su MJ, Yang YF, Liu YB, Liang HC, Wu CC, Lee YT 2004. Effect of hypercholesterolemia on myocardial function in New Zealand white rabbits. J Biomed Sci; 11(6):829-37. PMID: 15591780.

Mihos CG, Salas MJ, Santana O 2010. The pleiotropic effects of the hydroxy-methyl-glutaryl-CoA reductase inhibitors in cardiovascular disease: a comprehensive review. Cardiol Rev; 18(6):298-304. PMID: 20926939.

Mosca L, Biviano A 2002. National Cholesterol Education Program. Lipid-lowering therapies in the management of acute coronary syndromes. Curr Cardiol Rep; 4(4):320-6. PMID: 1205227952.

Oliveira TV, Maniero F, Santos MH, Bydlowski SP, Maranhão RC 2011. Impact of high cholesterol intake on tissue cholesterol content and lipid transfers to high-density lipoprotein. Nutrition; 27(6):713-8. Epub 2010 Oct 8. PMID: 20934854.

Osipov RM, Bianchi C, Feng J, Clements RT, Liu Y, Robich MP, Glazer HP, Sodha NR, Sellke FW 2009. Effect of hypercholesterolemia on myocardial necrosis and apoptosis in the setting of ischemia-reperfusion. Circulation; 120(11 Suppl):S22-30. PMID: 19752371.

Palaniswamy C, Selvaraj DR, Selvaraj T, Sukhija R 2010. Mechanisms underlying pleiotropic effects of statins. Am J Ther; 17(1):75-8. PMID: 19451808.

Penna C, Mancardi D, Raimondo S, Geuna S, Pagliaro P 2008. The paradigm of postconditioning to protect the heart. J Cell Mol Med; 12:435-458. PMID: 18182064.

Peterson DW, Napolitano CA, Griffith DW Jr 1980. Spontaneous mechanical alternans in papillary muscles from atherosclerotic rabbits. Am J Physiol; 239:H674-H680. PMID: 7435642.

Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, Carnethon MR, Dai S, de Simone G, Ford ES 2011. Heart disease and stroke statistics--2011 update: a report from the American Heart Association. Circulation; 123(6):e240. PMID 21160056

Rubinstein J, Pelosi A, Vedre A, Kotaru P, Abela GS 2009. Hypercholesterolemia and myocardial function evaluated via tissue doppler imaging. Cardiovasc Ultrasound; 7:56. PMID:19943937.

Sakamoto S, Kashiki M, Imai N, Liang CS, Hood WB Jr 1991. Effects of short-term, diet-induced hypercholesterolemia on systemic hemodynamics, myocardial blood flow, and infarct size in awake dogs with acute myocardial infarction. Circulation; 84:378-386. PMID: 2060108.

Satoh K, Takaguri A, Itagaki M, Kano S, Ichihara K 2008. Effects of rosuvastatin and pitavastatin on ischemia-induced myocardial stunning in dogs. J Pharmacol Sci; 106(4):593-9.PMID: 18403900.

Scalia R, Gooszen ME, Jones SP, Hoffmeyer M, Rimmer DM 3rd, Trocha SD, Huang PL, Smith MB, Lefer AM, Lefer DJ 2001. Simvastatin exerts both anti-inflammatory and cardioprotective effects in apolipoprotein E-deficient mice. Circulation; 103(21):2598-603. PMID: 11382730.

Schwartz LM, Lagranha CJ 2006. Ischemic postconditioning during reperfusion activates Akt and ERK without protecting against lethal myocardial ischemia-reperfusion injury in pigs. Am J Physiol Heart Circ Physiol; 290:H1011–1018. PMID: 16214840.

Shah KR, Kolar F, Beamish RE, Dhalla NS 1990. Depression in cardiac contractile force induced by cholesterol. Cardioscience; 1:255-259. PMID: 2151760.

Shkliar TF, Bliakhman FA, Aretinskiĭ VB 1991. An assessment of myocardial contractility in experimental atherosclerosis. Fiziol Zh SSSR Im I M Sechenova; 77(11): 48-54. PMID: 1668667.

Song G, Tian H, Qin S, Sun X, Yao S, Zong C, Luo Y, Liu J, Yu Y, Sang H, Wang X 2011. Hydrogen decreases athero-susceptibility in apolipoprotein B-containing lipoproteins and aorta of apolipoprotein E knockout mice. Atherosclerosis; Dec 13 PMID: 22209213.

Szilvassy Z, Ferdinandy P, Nagy I, Jakab I, Koltai M 1997. The effect of continuous versus intermittent treatment with transdermal nitroglycerin on pacing-induced preconditioning in conscious rabbits. Br J Pharmacol; 121:491-496. PMID: 9179391.

Szucs G, Bester DJ, Kupai K, Csont T, Csonka C, Esterhuyse AJ, Ferdinandy P, Van Rooyen J 2011. Dietary red palm oil supplementation decreases infarct size in cholesterol fed rats. Lipids Health Dis; 10:103. PMID: 21689423.

Talini E, Di Bello V, Bianchi C, Palagi C, Delle Donne MG, Penno G, Nardi C, Canale ML, Del Prato S, Mariani M, Miccoli R 2008. Early impairment of left ventricular function in hypercholesterolemia and its reversibility after short term treatment with rosuvastatin A preliminary echocardiographic study. Atherosclerosis; 197(1):346-54. PMID: 17631295.

Tang XL, Stein AB, Shirk G, Bolli R 2004. Hypercholesterolemia blunts NO donor-induced late preconditioning against myocardial infarction in conscious rabbits. Basic Res Cardiol; 99:395-403. PMID: 15372283.

Thuc LC, Teshima Y, Takahashi N, Nagano-Torigoe Y, Ezaki K, Yufu K, Nakagawa M, Hara M, Saikawa T 2010. Mitochondrial K(ATP) channels-derived reactive oxygen species activate pro-survival pathway in pravastatin-induced cardioprotection.Apoptosis;15(6):669-78. PMID: 20151195.

Tiefenbacher CP, Kapitza J, Dietz V 2003. Reduction of myocardial infarct size by fluvastatin. Am J Physiol Heart Circ Physiol; 285:H59-H64. PMID: 12623790.

Tilton RG, Cole PA, Zions JD, Daugherty A, Larson KB, Sutera SP, Kilo C, Williamson JR 1987. Increased ischemia-reperfusion injury to the heart associated with short-term, diet-induced hypercholesterolemia in rabbits. Circ Res; 60(4):551-9. PMID: 3594739.

Toleikis PM, Tomlinson CW 1987. Improved functional recovery of the atherosclerotic rabbit heart subjected to normothermic global ischemia. Can J Cardiol; 3:345-350. PMID: 3427533.

Ueda Y, Kitakaze M, Komamura K, Minamino T, Asanuma H, Sato H, Kuzuya T, Takeda H, Hori M 1999. Pravastatin restored the infarct size-limiting effect of ischemic preconditioning blunted by hypercholesterolemia in the rabbit model of myocardial infarction. J Am Coll Cardiol; 34:2120-2125. PMID: 10588233.

Van Craeyveld E, Jacobs F, Gordts SC, De Geest B 2011. Low-density lipoprotein receptor gene transfer in hypercholesterolemic mice improves cardiac function after myocardial infarction. Gene Ther; Oct 6. doi: 10.1038/gt.2011.147

Van de Velde M, DeWolff M, Leather HA, Wouters PF 2000. Effects of lipids on the functional and metabolic recovery from global myocardial stunning in isolated rabbit hearts. Cardiovasc Res; 48:129-137. PMID: 11033115.

Vinten-Johansen J, Yellon DM, Opie LH 2005. Postconditioning: a simple, clinically applicable procedure to improve revascularization in acute myocardial infarction. Circulation; 112:2085-2088. PMID: 16203924.

Wang TD, Chen WJ, Su SS, Lo SC, Lin WW, Lee YT 2002. Increased cardiomyocyte apoptosis following ischemia and reperfusion in diet-induced hypercholesterolemia: relation to Bcl-2 and Bax proteins and caspase-3 activity. Lipids; 37: 385-394. PMID: 12030319.

Wang W, Schulze CJ, Suarez-Pinzon WL, Dyck JR, Sawicki G, Schulz R 2002. Intracellular action of matrix metalloproteinase-2 accounts for acute myocardial ischemia and reperfusion injury. Circulation; 106:1543–1549. PMID: 12234962.

Zhai Y, Gao X, Wu Q, Peng L, Lin J, Zuo Z. 2008. Fluvastatin decreases cardiac fibrosis possibly through regulation of TGF-beta(1)/Smad 7 expression in the spontaneously hypertensive rats. Eur J Pharmacol; 587(1-3):196-203. Epub 2008 Mar 15. PMID: 18430418.

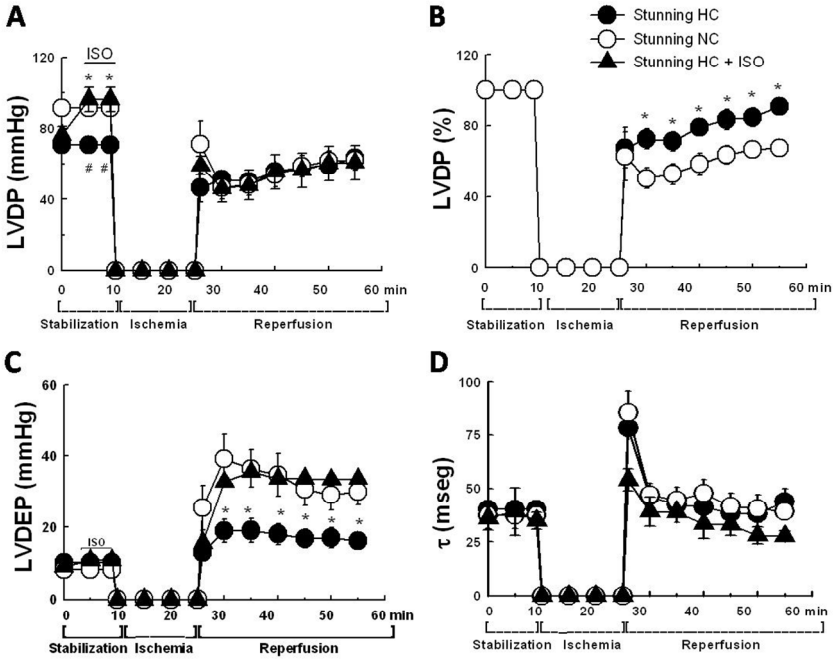
Legends

Figure 1: Panel A and B showed left ventricular developed pressure in absolute and percentage values, respectively. It was observed that the beneficial effect on the stunned myocardium obtained with cholesterol was abolished (Panel A), when the preischemic inotropic state was matched with isoproterenol (iso) administration (Panel B). Panel C showed the attenuation in the diastolic stiffness through left ventricular end diastolic pressure (LVEDP) in hypercholesterolemic animals, but when isoproterenol was administered, this attenuation of the myocardial stiffness was abolished. Panel D showed τ (msec) represents constant time of the left ventricular pressure decay during the relaxation phase isovolumic and is an index of isovolumic relaxation behavior. We did not detected any change among the studied groups. *p<0.05 versus normal diet; # p<0.05 versus cholesterol+Iso. Stabiliz: Stabilization. Iso: Isoproterenol.

Figure 2: Schematic diagram of the high-cholesterol diet effects on ventricular function behavior and myocardial stunning. MVO_2: Oxygen Consumption.

Figure 3: Panel A showed the increase of infarct size in hypercholesterolemic size compared to normocholesterolemic animals. Panel B showed that ischemic preconditioning significantly decreased infarct size in the hearts of normocholesterolemic and hypercholesterolemic animals. Panel C showed that rosuvastatin during reperfusión significantly reduced infarct size in normocholesterolemic and hypercholesterolemic animals. Panel D showed the administration of doxycycline during reperfusión significantly reduced infarct size in normocholesterolemic and hypercholesterolemic animals. ○ : represent single experiments; ● represent the mean ± standard error. *p<0.05 versus normocholesterolemic control, # p<0.05 versus hypercholesterolemic control. Precon: preconditioning. Rosu: Rosuvastatin. Doxy: Doxycycline.

Figure 4: Panel A showed the effect of acute pretreatment with simvastatin (S) and pravastatin (P) on postischemic recovery of left ventricular developed pressure (LVDP), left ventricular diastolic pressure (LVDIP; Panel B), size of infarction (IS) expressed as percentage of left ventricular area (LV; Panel C) and Panel D showed the severity of ventricular arrhythmias in the hearts of normocholesterolemic rats. Empty bars – untreated control hearts, filled bars – S-treated hearts, hatched bars – P-treated hearts. Values are means ± S.E.M. from 10-12 hearts per group. *$p<0.05$ vs. untreated control group; # $p<0.05$ vs. simvastatin-pretreated group. (From S. Carnicka et al. 2011, reproduced with permission of Physiol. Res., Vol. 60, p. 827, © 2011 Physiological Research.)



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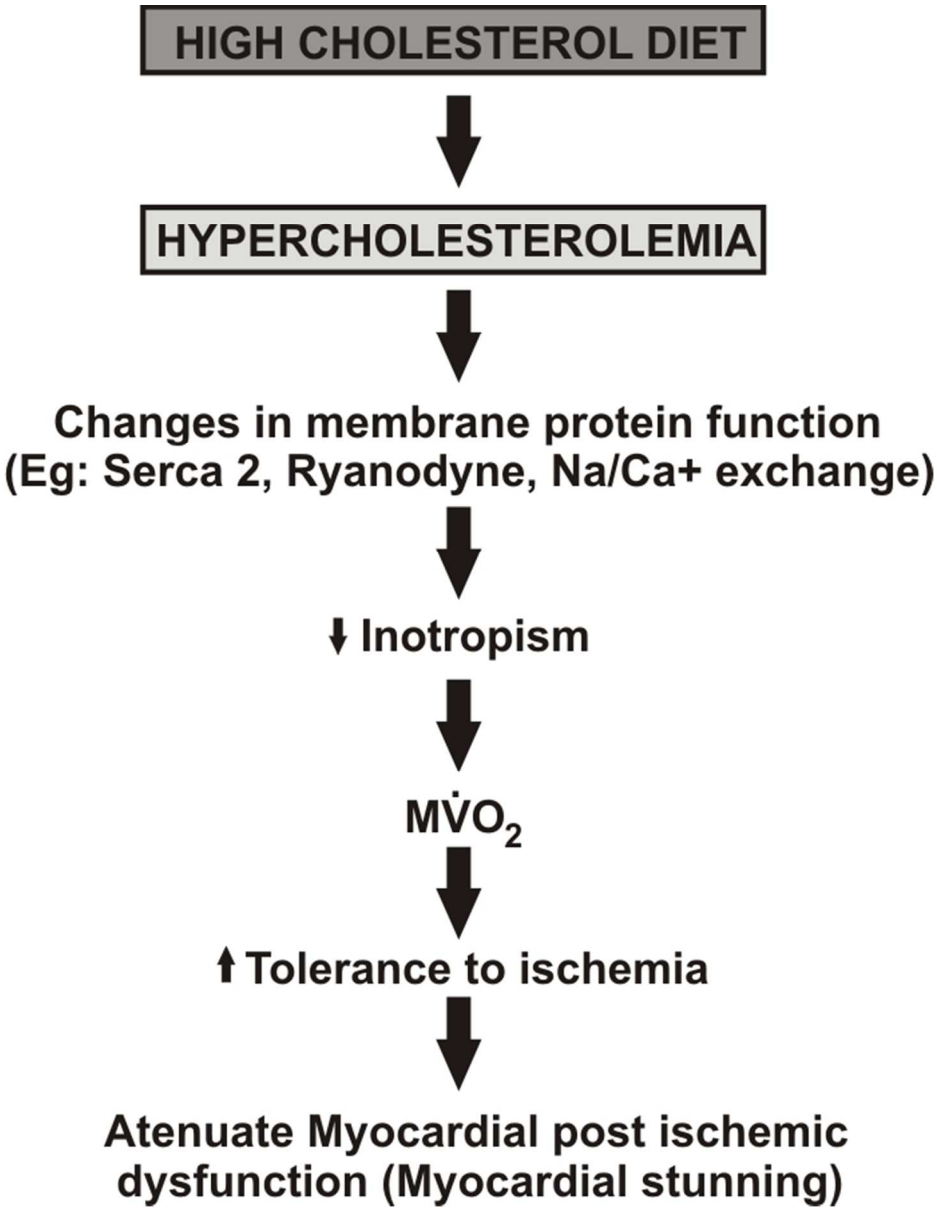

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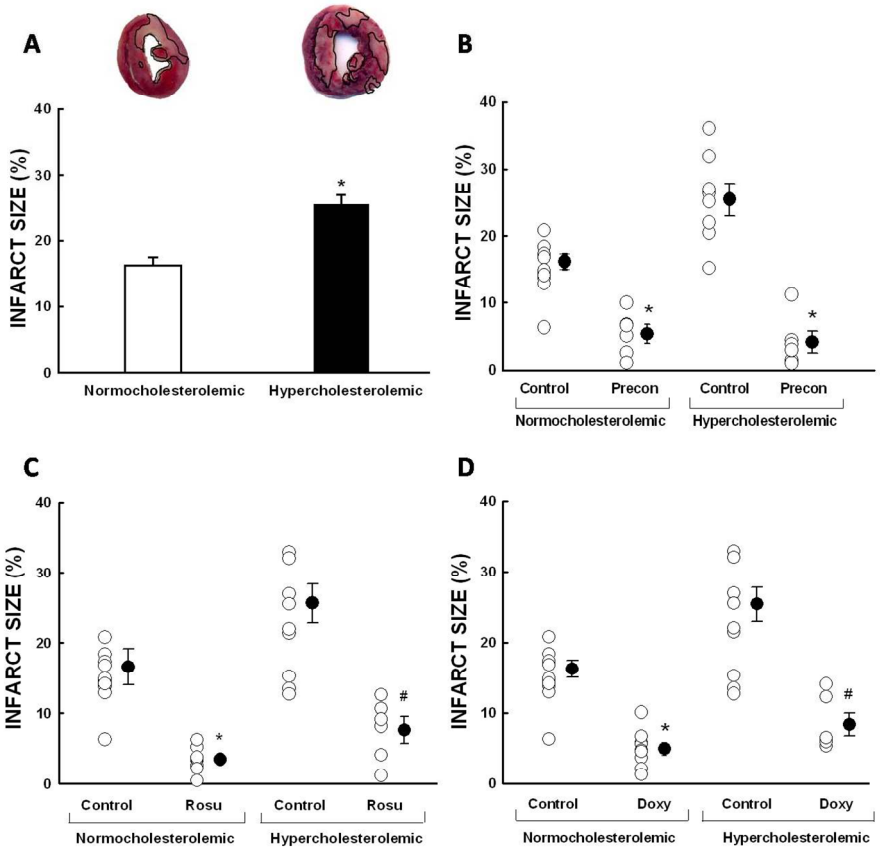

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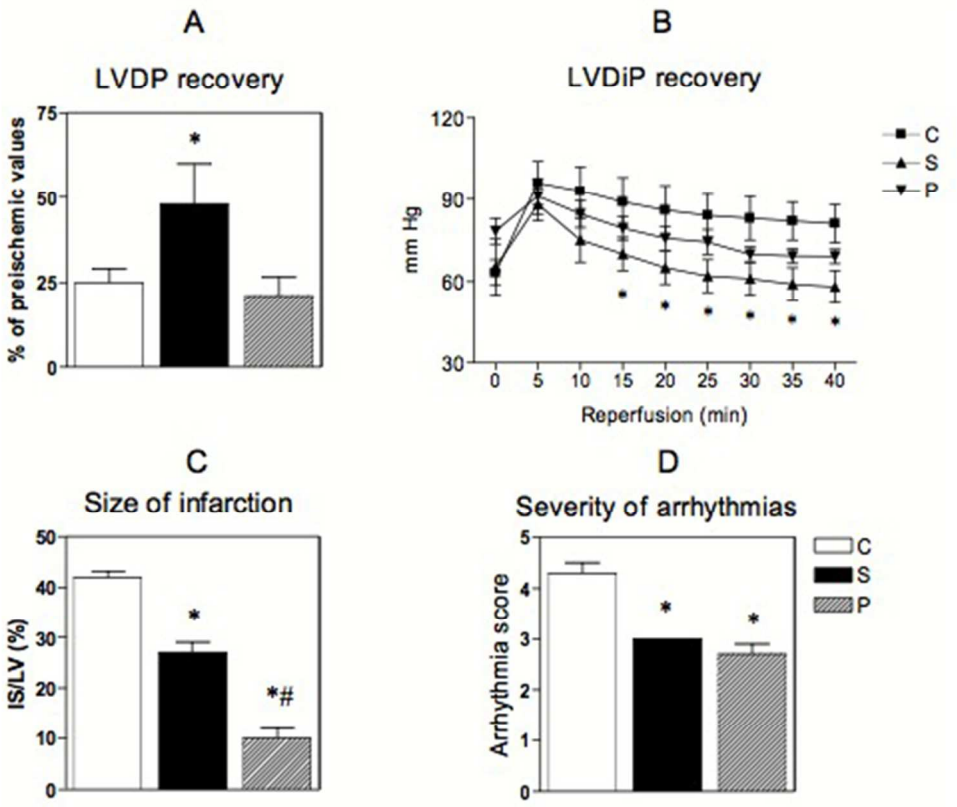

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