European Review for Medical and Pharmacological Sciences 2019; 23: 4439-4447

Pulsatile antagonism on bradykinin 2-receptor (BK2R) by icatibant triggers the most effective kinin-dependent post-conditioning on rat hearts

L. SGARRA, C. COCO, M. MONTAGNANI, M.A. POTENZA

Department of Biomedical Sciences and Human Oncology, University of Bari "Aldo Moro", Bari, Italy

Abstract. – OBJECTIVE: Pharmacological post-conditioning (PC) by intermittent but not continuous administration of exogenous bradykinin (BK) reduces ischemia/reperfusion (I/R) injury via the Reperfusion Injury Salvage Kinase (RISK) pathway activation. We evaluated whether intermittent administration with icatibant (HOE140), a BK2R antagonist, may represent an effective PC strategy, with the advantage of limiting the potential risks of supra-physiologic BK activity.

MATERIALS AND METHODS: Hearts from male Sprague-Dawley (SD) rats on a Langendorff system were exposed to I/R injury (30/120 min). BK (100 nM) and HOE140 (1 μM) were administered post-ischemically during the first 3 min of reperfusion, under continuous or intermittent infusion (10 s/each). Hearts were randomly assigned to 5 groups: 1) I/R alone (n=5); 2) continuous HOE140 (cHOE n=6); 3) intermittent HOE140 (iHOE n=6); 4) continuous BK (cBK n=6); 5) intermittent BK (iBK n=6). End-diastolic left ventricular pressure (LVEDP), developed left ventricular pressure (dLVP) and coronary flow (CF) were monitored throughout reperfusion. Left ventricular infarct mass (IM) was quantified together with the phosphorylated levels of Akt and GSK3β (RISK pathway kinases) at the end of reperfusion.

RESULTS: IM was not significantly changed in cBK or cHOE groups (vs. I/R). Conversely, both iBK and iHOE groups showed a significant limitation in IM (vs. I/R, $p<0.05$, $p<0.01$, respectively). Akt and GSK3β phosphorylation levels were higher in iBK and iHOE groups (*vs.* I/R, $p<0.05$). When compared to I/R group, both LVEDP values ($p<0.05$, first 60-min reperfusion), as well as dLVP values ($p<0.01$) were improved only in iHOE group. CF values did not vary among all groups.

CONCLUSIONS: In isolated rat hearts, intermittent modulation of the endogenous kallikrein-kinin system by a selective BK2R antagonist mediates PC cardioprotection via RISK signaling.

Key Words:
Post-conditioning strategies, Bradykinin, HOE140, Icatibant.

Introduction

Clinical outcomes in patients surviving an acute myocardial infarction (AMI) highlight the importance of treatments aimed at reducing the extent of cardiac cell death in response to initial ischemia and subsequent restoration of blood supply (ischemia and reperfusion injury, I/R). Following the initial observation of Murry et al[1], pioneering the protective effect of intermittent ischemia, a wide number of conditioning strategies have been proposed to increase the heart resilience under I/R injury[2], in experimental[3-5] and human[6] studies. Both pre-conditioning and post-conditioning (PC) strategies trigger an adaptive response associated with cardioprotection[7-10] by facilitating the appropriate time-release of intracellular mediators into the coronary circulation[11] and activating signaling mechanisms, collectively called reperfusion injury salvage kinase (RISK)[12] and survivor activating factor enhancement (SAFE)[13] pathways. Despite the increased understanding of the causes and mechanisms underlying the I/R injury, this complex issue is far from clear, and investigation on potential strategies to limit heart damage is still ongoing. Among the endogenous mediators implicated in cardioprotection, the role of bradykinin (BK) has been initially suggested by studies revealing the importance of the kinin-kallikrein system on the regulation of cadiovascular homeostasis[14] and progressively pointed out by the increasing evidence of the BK-mediated contribution to the activity of angiotensin-converting enzyme inhibitors (ACE-I). Indeed, pharmacological pre-conditioning with ACE-I enhances cardioprotection, in part, by preserving BK from degradation and, in some cases, by directly activating BK receptors[15-18]. Similarly, the finding that ischemic PC triggers cardioprotection via BK-dependent signaling mechanisms[19,20] supports

Corresponding Author: Maria Assunta Potenza, MD; email: mariaassunta.potenza@uniba.it

the observation that PC with either exogenous BK or ACE-I confers an overlapping pattern of hemodynamic and metabolic rearrangements in the myocardial cells[21].

Over the past several years, the use of pharmacological BK agonists/antagonists and the availability of genetically engineered animals has allowed the characterization of the two BK receptor types, BK1R[22,23], and BK2R[24,25]. In more recent years, preclinical and clinical studies have repeatedly suggested that the constitutive BK2R, when activated, seems to play an important role in the process of ischemic PC that limits the I/R injury of the myocardium[26]. Conversely BK1R, mostly unexpressed under normal conditions but induced by a variety of pathological conditions (including the presence of toxins, cytokines or inflammatory mediators resulting from tissue injury), appears to be implicated in mainly harmful effects of BK.

Notably, consistent with the observation that the heart releases kinins that accumulate in an intermittent manner during the brief I/R periods of PC, it has been reported that exogenous BK exerts cardioprotection when intermittently administered during the early reperfusion, whereas it does not enhance myocardial tolerance if continuously infused[27]. Accordingly, we observed that PC with continuous infusion of the specific BK2R antagonist HOE140 (icatibant) abolished BK-mediated losartan protection[28]. These findings support the notion that, besides specific recruitment of BK receptor subtypes, the BK system plays a key role in PC protection only when modulated in an intermittent manner. Thus, timely activation and release of downstream mediators is an essential condition for putting the heart into a protected state.

The concept that PC cardioprotection obtained with exogenous BK requires a pulsatile approach suggests that strategies creating an oscillatory variation in endogenous BK levels may result equally effective, with the potential advantage of limiting the unnecessary stimulation of BK receptors by supra-physiological BK levels. Indeed, unselective potentiation of BK system may lead to mixed results, as activation of the BK1R appears to have predominantly noxious effects while excessive activation of BK2R, although predominantly cardioprotective, may also induce some systemic side effects. Thus, the aim of this study was to evaluate whether intermittent administration of HOE140 – icatibant may represent an effective post-conditioning strategy, alternative to exogenous BK administration.

Materials and Methods

Animal Experiments

Animal procedures were performed under authorization obtained by the Committee on the Ethics of Animal Experiments of the University of Bari and by the Ministry of Health (Italian Government, prot.n. 216/2016-PR). Adult male Sprague-Dawley (SD) rats weighing 250-300 g were obtained from Envigo (Udine, Italy) and housed at the Department animal facility. Rats randomly assigned to protocols described below were anesthetized with sodium pentobarbital (80 mg/kg body weight i.p.), heparinized (400 UI/100g body weight i.p.) and euthanized by cervical dislocation. All efforts were made to minimize animal suffering.

Drugs

HOE-140 and BK were from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions of BK (10 mM) and HOE140 (0.2 mM) were prepared in distilled water. Final dilutions were prepared in modified Krebs-Henseleit solution [composed of (mmol/l): 118.5 NaCl; 4.7 KCl; 1.2 $MgSO_4$; 1.2 KH_2PO_4; 1.25 $CaCl_2(H_2O)$; 25 $NaHCO_3$; 11 glucose] immediately before use. For each drug used, concentration was chosen according to literature data[27,29].

Myocardial Function and Infarct Size in Isolated Hearts

The excised hearts were immediately mounted on a Langendorff perfusion system (Radnoti LLC, Covina, CA, USA). Heart isolation technique and *ex-vivo* perfusion procedure have been previously described and are available at[28]. In our isovolumetric model of isolated heart, the perfusion pressure (PP) was kept constant at 80 mmHg for the whole experimental procedure. Left ventricular systolic (LVSP) and end-diastolic (LVEDP) pressures were recorded starting from LVEDP values set to 5-10 mmHg at the beginning of the experimental procedure. The timed collection of coronary effluent provided the measurement of coronary flow. Left ventricular developed pressure (dLVP) was calculated as dLVP = LVSP – LVEDP. To obtain global ischemia, the inflow tubing to the hearts was clamped for 30 min, and then the hearts reperfused for 120 min. After stabilization, hearts were not evaluated in case of unstable contractile function, LVSP outside the range of 60-160 mmHg, heart rate below 240 beats min^{-1} or appearance of severe arrhythmia.

All parameters of left ventricular function, as well as coronary flow were recorded twice before ischemia, and at min 5, 15, 30, 45, 60, 90, and 120 of reperfusion using a 4-channel PowerLab system (ADInstruments, UK), and analyzed using LabChart 7 Pro Software (ADInstruments, UK).

The infarct area extent was measured at the end of reperfusion in hearts stained with freshly prepared 2,3,5 triphenyltetrazolium chloride (TTC 1% w/v phosphate buffer pH 7.4, 37°C, 20 min), as previously described[28]. The infarct area on each color image (TTC unstained) was measured by planimetry (Image-Tool 2.0 Software NIH, Bethesda, MD, USA). The area at risk (AAR, representing total infarct area with respect to total muscle mass) was calculated by the sum of individual slice weights according to the following formula: (*AIn/AARn*) x (*Wn/Wtotal*), where AI is the infarct area of each slice (n = 7), Wn is the weight of the respective section (n) and Wtotal is the sum of all slice weights.

Experimental Protocol

In all groups, hearts were subjected to 20 min of stabilization, 30 min of global no-flow ischemia and subsequent 120 min of reperfusion. Post-ischemic hearts were treated with different drugs during the first 3 min of reperfusion, using a second reservoir. For intermittently administered drugs, the protocol alternated 9 cycles of 10 s/each with Krebs+drug to 9 cycles of 10 s/each with Krebs+vehicle. Temperature, oxygen tension, and perfusion pressure between reservoirs did not vary during the whole experimental procedure.

The following treatments were performed: ischemia/reperfusion (I/R) (vehicle-treated; n= 5); intermittent perfusion with bradykinin (100 nM[27]) (iBK, n = 6); continuous perfusion with bradykinin (100 nM) (cBK, n = 6); intermittent perfusion with HOE-140 (1 μM[29]) (iHOE, n = 6); continuous perfusion with HOE-140 (1 μM) (cHOE, n = 6).

Western Blot Analysis

The activation of signaling pathways was evaluated at the early reperfusion time (15 min), in 3 additional hearts/group for I/R, iBK, cBK, iHOE, and cHOE treatments and in hearts from control animals (CTRL, not subjected to I/R)[28]. Samples of the left ventricular tissue were freeze-clamped in liquid nitrogen, homogenized on ice-cold radioimmunoprecipitation assay (RIPA) lysis buffer[28], and then centrifuged (4°C for 15 min at 13.000 g). The protein level was determined by Bradford's method[30].

Equal amounts of protein (100 μg) were separated by 10% of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and subjected to immunoblotting with primary antibodies (dilution 1:1000) targeting Akt, p-Akt, GSK3β, p-GSK3β (Cell Signaling Technology, Danvers, MA, USA) and with appropriate HRP-linked secondary antibodies (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) (1:3000). Immunoblotting results were visualized by Molecular Imager ChemiDoc XRS System (Bio-Rad Laboratories, Hercules, CA, USA). Images were captured with Quantity One Software (Bio-Rad Laboratories, Hercules, CA, USA) and blots quantified by scanning densitometry (ImageJ, NIH, Bethesda, MD, USA).

Statistical Analysis

Based on the power analysis prospectively performed, a sample size of 5 (in each group) was sufficient to detect 10% differences in infarct area extent ($\alpha = 0.05$) with a power of 0.80. Results are expressed as mean ± standard error (SE) of *n* experiments (*n* = number of rats).

Statistical analysis was performed with a two-way ANOVA for repeated measures. Where appropriate, a one-factor ANOVA was used followed by the Bonferroni correction. A *p*-value less than 0.05 was considered to detect a statistically significant difference. Statistica Release 7 (Statoft Institute Inc.) was used to perform all analysis.

Results

Intermittent HOE Administration Partially Ameliorates Post-Ischemic Left Ventricular Recovery in Rat Hearts

To evaluate whether the intermittent administration of HOE140 – icatibant may represent an effective post-conditioning strategy, alternative to exogenous BK administration, parameters estimating left ventricular function in hearts infused with BK during the first 3 min of reperfusion in intermittent (iBK group) or continuous (cBK group) manner were compared to those obtained in hearts under intermittent (iHOE group) or continuous (cHOE group) HOE administration, and with respect to hearts infused with vehicle alone (3 mL/1 min) (I/R group). LVEDP represents an index of left ventricular contractility, whose values increase proportionally to the de-

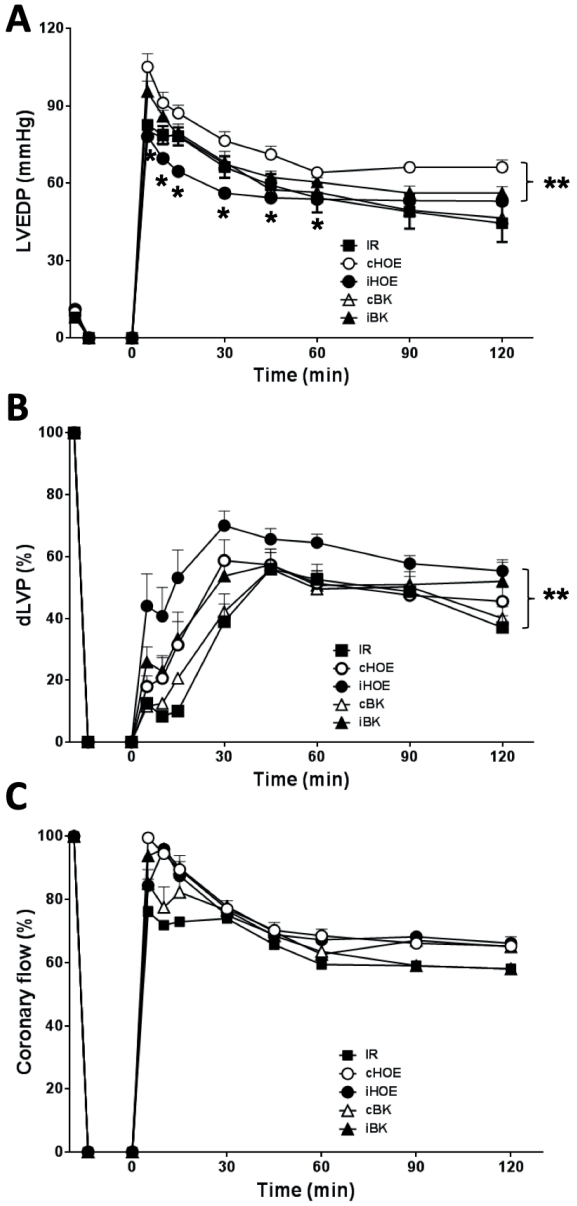

Figure 1. Intermittent HOE administration partially ameliorates post-ischemic left ventricular recovery in rat hearts. Hearts infused with vehicle at the onset of reperfusion (I/R group) were compared to hearts achieved by pharmacological post-conditioning with either BK or HOE, infused under intermittent (iBK or iHOE groups) or continuous (cBK or cHOE groups) administration. Results are expressed as mean ± SEM of 5 independent experiments for each group. Two-way repeated measures ANOVA was employed to determine the main effect of time, group and time by group interaction. ***A,*** Diastolic function. Left ventricular end diastolic pressure (LVEDP) for each group during 120 min of reperfusion (**p<0.01 iHOE *vs.* cHOE); (*p<0.05 at 5,10,15,45,60 min iHOE *vs.* I/R group). ***B,*** Systolic function. Percent variation of developed left ventricular pressure (dLVP) for each group during 120 min of reperfusion (**p<0.01 iHOE *vs.* I/R). ***C,*** Percent variation of coronary flow for each group during 120 min of reperfusion.

gree of ventricular failure. Consistent with previous results[28], LVEDP obtained in hearts exposed to cBK were not significantly different from the values obtained in the I/R group during the whole reperfusion time (120 min) (Figure 1A). BK intermittently administered (iBK group) slightly but not significantly decreased LVEDP during the whole reperfusion period, with LVEDP values substantially overlapping those obtained in hearts from I/R group. Continuous perfusion with HOE (cHOE group) maintained LVEDP values slightly higher than in I/R group (p = 0.09); on the other hand, intermittent HOE administration (iHOE group) lowered LVEDP during the whole reperfusion, with a significant difference *vs.* respective values of the I/R group during the first 60 min of reperfusion (p<0.05) and *vs.* the cHOE group during the total 120 min of reperfusion (p<0.01). Values of dLVP overlapped in both cBK and iBK groups, and did not significantly differ with respect to the I/R group (Figure 1B). Continuous administration of HOE (cHOE group) did not worsen dLVP (*vs.* I/R group); once again, HOE administered in the intermittent manner (iHOE group) significantly improved dLVP (p<0.01 *vs.* I/R group, Figure 1B). Coronary flow was unchanged among experimental groups (Figure 1C). Indeed, none of the drug infused (BK or HOE) with whichever procedure (intermittent *vs.* continuous administration) modified the volume efflux suggesting that, under our experimental conditions, the contribution of the BK system to coronary perfusion is negligible.

Post-Ischemic Intermittent HOE Administration Limits the Infarct Extension in Rat Hearts

Besides functional parameters, the most reliable evaluation of whichever cardioprotective strategy relies on the infarct area extent. Thus, at the end of reperfusion, the percentage of the ischemic area was compared among hearts. Not surprisingly, intermittent (but not continuous) BK administration resulted in a significant reduction of the infarct extension when compared to I/R group (p<0.05). Consistent with the partially improved left ventricular performance, the infarct size was significantly smaller in hearts from iHOE group (p<0.01 *vs.* I/R group) (Figure 2A). Continuous infusion with BK (cBK group) did not significantly reduce the extent of infarct area (*vs.* I/R, p=0.79) (Figure 2A); continuous infusion with HOE did not worsen this parameter (*vs.* I/R, p=0.85) (Figure 2A).

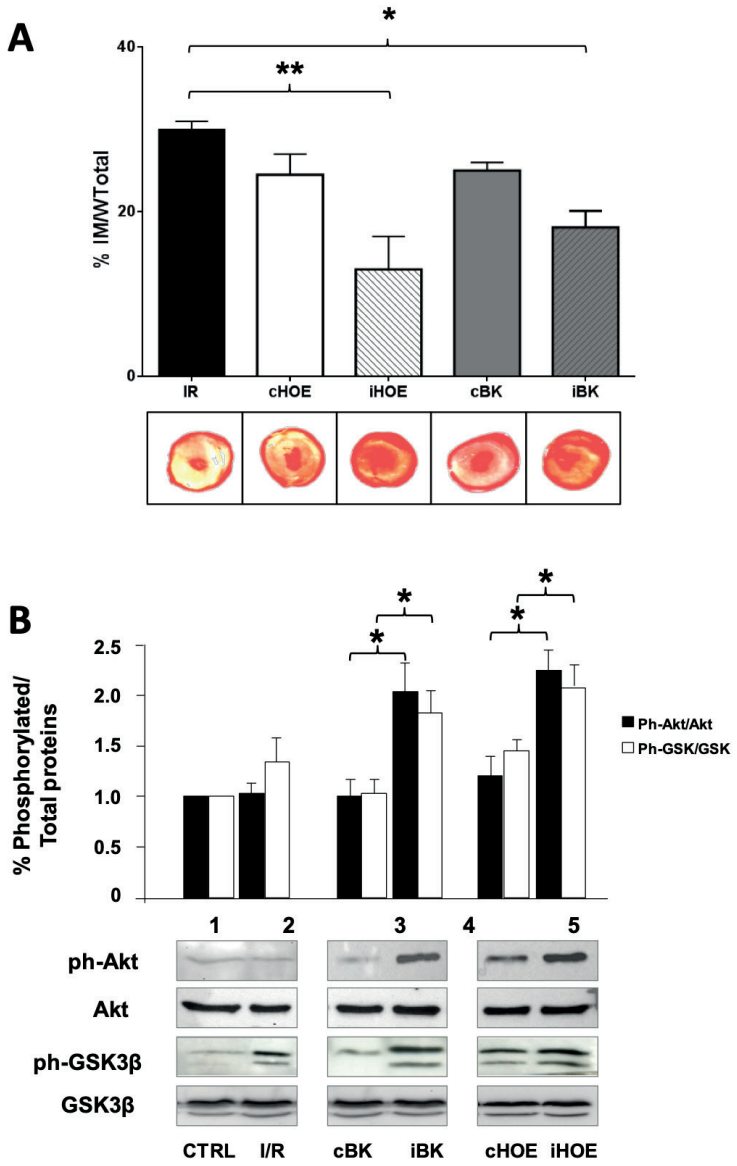

Figure 2. Cardioprotection by intermittent BK or HOE administration involves activation of RISK pathway. ***A,*** Upper panel. Quantification of necrotic tissue at the end of the reperfusion interval, expressed as percentage of the left ventricular mass, in hearts infused with vehicle alone (I/R) compared to hearts exposed to various pharmacological post-conditioning. Statistical differences between groups were evaluated by one-factor ANOVA followed by Bonferroni correction. Values are expressed as mean ± SEM (**$p < 0.01$; *$p < 0.05$). ***A,*** Lower panel. Representative images of left ventricular sections quantified in the panel above are shown. Hearts were cut into transverse slices and stained with TTC to differentiate necrotic (unstained) from viable (red) myocardial tissue. ***B,*** Upper panel. Lysates obtained after 15 min of reperfusion from hearts exposed to different pharmacological treatments were immunoblotted for phosphorylated and total isoforms of the indicated proteins. For each experimental condition, the ratio of phosphorylated/total Akt and GSK3β proteins are expressed as the mean ± SEM of at least 3 independent experiments (each run in triplicate). Statistical differences between groups were evaluated by one-factor ANOVA followed by Bonferroni correction (*$p< 0.05$). ***B,*** Lower panel. Representative immunoblots of total and phosphorylated Akt and GSK3β proteins expression quantified in upper panel are shown.

Post-Ischemic Intermittent HOE Administration Activates the Cardioprotective RISK Pathway

To explore whether BK and HOE might differentially stimulate intracellular RISK signaling pathways, the phosphorylation state of Akt (ser473), and GSK3β (ser9) downstream was evaluated 15 min after reperfusion in homogenates from hearts exposed to our post-ischemic treatments, and compared to hearts from animals not exposed to I/R injury (CTRL group) (Figure 2B). Protein expression of Akt and GSK3β did not

vary among groups. With respect to hearts of the I/R group, levels of phosphorylated Akt did not significantly change in hearts exposed to continuous infusion with either BK (cBK group) or HOE (cHOE group) (Figure 2B). Conversely, phosphorylation of Akt was higher in hearts from the iBK group ($p<0.05$ *vs.* I/R group) and from the iHOE group ($p<0.05$ *vs.* I/R group). Thus, for both BK and HOE treatments, the intermittent administration was able to significantly increase Akt phosphorylation, whereas the continuous infusion was not (cBK *vs.* iBK and cHOE *vs.* iHOE, $p<0.02$, for both). Analogous results were observed for the phosphorylated levels of GSK3β (cBK *vs.* iBK; cHOE *vs.* iHOE, $p<0.02$, for both) (Figure 2B). Since the switching perfusion procedure did not exert *per se* any change in signaling explored (I/R *vs.* CTRL, no significant difference), the findings described could be *bona fide* ascribed to the specific drug treatments.

Discussion

The tissue-protective actions of BK in hearts exposed to acute myocardial infarction are firmly established[31]. BK exerts the majority of vasodilator and insulin-sensitizing effects through the constitutively expressed BK2R, whereas activation of inducible BK1R may rather cause harmful consequences. Since both BK1R and BK2R are up-regulated under myocardial injury[32,33], any exogenous BK administration will likely act on both receptor subtypes, with possibly detrimental –when not adverse– outcomes. Preliminary data on highly selective BK2R agonists[34] have shown promising results[26], but their safety and long-term efficacy needs further evaluation. Notably, the surplus activity on BK2R is not without risks as well, since BK2R triggers the typical symptoms of BK-mediated inflammation and severe side effects such as angioedema, as reported in patients treated with ACE-I[35]. Indeed, the selective BK2R antagonist HOE-140 (icatibant), currently licensed to treat hereditary angioedema, has been recently proposed as emergency treatment for ACE-I-induced angioedema[36]. These concepts, together with the notion that exogenous BK confers myocardial protection only when administered intermittently, suggests that therapeutic strategies variating endogenous BK levels in a pulsatile fashion may result equally effective, with the advantage of limiting the potential risks of supra-physiological BK levels. Evaluation of parameters measuring post-ischemic left ventricular function corroborate this idea: in fact, with respect to I/R group, continuous activation of the BK system (cBK group) does not confer additional protection, while the continuous blockade of the BK2R (cHOE group) does not further worsen cardiac recovery. The finding that intermittent administration with BK did not significantly improve functional parameters was not particularly surprising, since the PC with exogenous BK, although successfully reducing infarct extension, does not substantially ameliorate ventricular recovery in the 2-h post-ischemic period[27]. Instead, in line with the hypothesis proposed in this study, left ventricular recovery was partially improved in hearts exposed to intermittent administration with the BK2R blocker HOE-140. Notably, in terms of effective reduction of infarct area extent, intermittent HOE-140 resulted in a slightly but significantly better performance than that obtained with exogenous intermittent BK. Downstream its receptors, BK activates a cascade of intracellular events culminating in the inhibition of the mitochondrial permeability transition pore (mPTP), which is believed to open during the first few minutes of reperfusion in response to mitochondrial calcium overload, burst of oxidative stress, reduced nitric oxide (NO) production, and ATP depletion[37,38]. In this signaling flow, the inhibition of mPTP opening is subsequent to the activation of kinases of the RISK pathway such as Akt, which in turn confers cardioprotection by phosphorylating the glycogen synthase kinase-3β (GSK3β)[12]. Since the intermittent administration (either with BK or with HOE-140) was able to significantly increase phosphorylation levels of both Akt and GSK3β, whereas the continuous infusion was not, our findings further support the hypothesis that pharmacological approaches modulating the endogenous BK levels in a pulsatile fashion may be as effective (and even more effective) than intermittent administration with exogenous BK. This apparently puzzling finding might be explained taking into account that intermittent HOE antagonism on BK2R allows accumulation and a subsequent burst of endogenous BK, which therefore may act on signaling cascades in appropriate physiological concentrations, and according to proper timing. Since parameters evaluating a potential drug treatment must carefully consider both effectiveness and safety, our findings imply that, by preserving endogenous BK levels, intermittent HOE administration may help to reduce the risks secondary to excessive

activation of systemic BK signaling on BK2R. In addition, in the absence of BK surplus, it is presumable that the endogenous concentrations of BK engage only an established number of BK receptors, therefore limiting the binding to BK1R and decreasing the subsequent potential harmful effects. Present findings have some limits: for example, the assessment of both BK1R and BK2R protein levels in hearts undergoing I/R injury and analysis of their specific contribution to heart recovery might elucidate the potential significance of our approach. Moreover, it is known that BK induces internalization and trafficking of BK2R in a concentration–and time– dependent manner , and that nuclear localization of BK2R contributes to transcriptional regulation of inducible NO synthase (iNOS) gene in some tissues[39]. iNOS is induced in the myocardium in response to hypoxia and ischemia[40,41] and its expression correlates positively with the severity of cardiac dysfunction[42]. Since BK affords myocardial protection through NO-dependent mechanisms[43], but an excessive NO production by iNOS is involved in oxidative stress[44], the evaluation on constitutive and inducible NO synthase expression in myocardial cells under BK or HOE administration may help to clarify the results obtained. At the moment, qualitative findings do not suggest a potential difference between these two treatments, but the data obtained so far are too preliminary to substantiate or refuse this possibility. Nonetheless, taking these limitations into account, we feel that our results may provide additional information and further insights on the role of BK system in heart recovery after ischemia which may be helpful to plan more effective and safe approaches in post-conditioning.

Conclusions

We observed that in isolated rat hearts exposed to I/R injury, post-conditioning with intermittent administration of HOE140 – icatibant ameliorates left ventricular function and reduces the extent of the ischemic area, via mechanisms involving the activation of RISK pathway kinase Akt and its downstream target GSK3β. Therapeutic strategies variating endogenous BK levels in a pulsatile fashion may result as effective as exogenous BK administration, with the advantage of limiting the potential risks of supra-physiological stimulation on both BK1 and BK2 receptors.

Conflict of interest

The authors declare no conflicts of interest.

Financial Support

This work was supported, in part, by the Italian Ministry of University (MIUR) research grant award (PRIN 2010YK7Z5K_008) to M.M. and, in part, by the University of Bari Research Funds (CUP H96J15001610005) to M.M.

Author Contributions

Conceived and designed the experiments: LS, MAP. Performed the experiments: MAP, CC, LS. Analyzed the data: MAP, LS, MM. Contributed reagents/materials/analysis tools: MM. Wrote the paper: MAP, MM.

References

1) Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 1986; 74: 1124-1136.
2) Brooks MJ, Andrews DT. Molecular mechanisms of ischemic conditioning: translation into patient outcomes. Future Cardiol 2013; 9: 549-568.
3) Li WN, Wu N, Shu WQ, Guan YE, Jia DL. The protective effect of fasudil pretreatment combined with ischemia postconditioning on myocardial ischemia/reperfusion injury in rats. Eur Rev Med Pharmacol Sci 2014; 18: 2748-2758.
4) Zhang ZX, Li H, He JS, Chu HJ, Zhang XT, Yin L. Remote ischemic postconditioning alleviates myocardial ischemia/reperfusion injury by up-regulating ALDH2. Eur Rev Med Pharmacol Sci 2018; 22: 6475-6484
5) Huang LH, Li J, Gu JP, Qu MX, Yu J, Wang ZY. Butorphanol attenuates myocardial ischemia reperfusion injury through inhibiting mitochondria-mediated apoptosis in mice. Eur Rev Med Pharmacol Sci 2018; 22: 1819-1824
6) Zhou FZ, Song W, Yin LH, Song ZF, Yang S, Yang FB, Liu JF, Song YG, Zhang HY, Zhang ZM. Effects of remote ischemic preconditioning on myocardial injury and endothelial function and prognosis after percutaneous coronary intervention in patients with acute coronary syndrome. Eur Rev Med Pharmacol Sci 2017; 21: 4642-4648
7) Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton RA, Vinten-Johansen J. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. Am J Physiol Heart Circ Physiol 2003; 285: H579-H588
8) Driamov S, Bellahcene M, Ziegler A, Barbosa V, Traub D, Butz S, Buser PT, Zaugg CE. Antiarrhythmic effect of ischemic preconditioning during low-flow ischemia. The role of bradykinin and sarcolemmal

versus mitochondrial ATP-sensitive K(+) channels. Basic Res Cardiol 2004; 99: 299-308.

9) Bice JS, Baxter GF. Postconditioning signalling in the heart: mechanisms and translatability. Br J Pharmacol 2015; 172: 1933-1946.

10) Potenza MA, Sgarra L, Nacci C, Leo V, De Salvia MA, Montagnani M. Activation of AMPK/SIRT1 axis is required for adiponectin-mediated preconditioning on myocardial ischemia-reperfusion (I/R) injury in rats. PLoS One 2019; 14: e0210654.

11) Heusch G. Cardioprotection: chances and challenges of its translation to the clinic. Lancet 2013; 381: 166-175.

12) Hausenloy DJ, Yellon DM. Reperfusion injury salvage kinase signalling: taking a RISK for cardioprotection. Heart Fail Rev 2007; 12: 217-234.

13) Hadebe N, Cour M, Lecour S. The SAFE pathway for cardioprotection: is this a promising target? Basic Res Cardiol 2018; 113: 9.

14) Greco AV, Porcelli G, Croxatio HR, Fedeli G, Ghirlanda G. [Arterial hypertension and urinary kallikrein]. Minerva Med 1974; 65: 3058-3062.

15) Liu X, Lukasova M, Zubakova R, Lewicka S, Hilgenfeldt U. A kallidin-like peptide is a protective cardiac kinin, released by ischaemic preconditioning of rat heart. Br J Pharmacol 2005; 146: 952-957.

16) Liu X, Lukasova M, Zubakova R, Lewicka S, Hilgenfeldt U. Kallidin-like peptide mediates the cardioprotective effect of the ACE inhibitor captopril against ischaemic reperfusion injury of rat heart. Br J Pharmacol 2006; 148: 825-832.

17) Miki T, Miura T, Ura N, Ogawa T, Suzuki K, Shimamoto K, Iimura O. Captopril potentiates the myocardial infarct size-limiting effect of ischemic preconditioning through bradykinin B2 receptor activation. J Am Coll Cardiol 1996; 28: 1616-1622.

18) Kimura S, Zhang GX, Nishiyama A, Shokoji T, Yao L, Fan YY, Rahman M, Suzuki T, Maeta H, Abe Y. Role of NAD(P)H oxidase- and mitochondria-derived reactive oxygen species in cardioprotection of ischemic reperfusion injury by angiotensin II. Hypertension 2005; 45: 860-866.

19) Ovize M, Baxter GF, Di Lisa F, Ferdinandy P, Garcia-Dorado D, Hausenloy DJ, Heusch G, Vinten-Johansen J, Yellon DM, Schulz R; Working Group of Cellular Biology of Heart of European Society of Cardiology. Postconditioning and protection from reperfusion injury: where do we stand? Position paper from the Working Group of Cellular Biology of the Heart of the European Society of Cardiology. Cardiovasc Res 2010; 87: 406-423.

20) Ferdinandy P, Hausenloy DJ, Heusch G, Baxter GF, Schulz R. Interaction of risk factors, comorbidities, and comedications with ischemia/reperfusion injury and cardioprotection by preconditioning, postconditioning, and remote conditioning. Pharmacol Rev 2014; 66: 1142-1174.

21) Linz W, Wiemer G, Gohlke P, Unger T, Schölkens BA. Contribution of kinins to the cardiovascular actions of angiotensin-converting enzyme inhibitors. Pharmacol Rev 1995; 47: 25-49.

22) Marceau F, Hess JF, Bachvarov DR. The B1 receptors for kinins. Pharmacol Rev 1998; 50: 357-386

23) Pesquero JB, Araujo RC, Heppenstall PA, Stucky CL, Silva JA Jr., Walther T, Oliveira SM, Pesquero JL, Paiva AC, Calixto JB, Lewin GR, Bader M. Hypoalgesia and altered inflammatory responses in mice lacking kinin B1 receptors. Proc Natl Acad Sci U S A 2000; 97: 8140-8145.

24) Regoli D, Barabé J. Pharmacology of bradykinin and related kinins. Pharmacol Rev 1980; 32: 1-46.

25) Borkowski JA, Ransom RW, Seabrook GR, Trumbauer M, Chen H, Hill RG, Strader CD, Hess JF. Targeted disruption of a B2 bradykinin receptor gene in mice eliminates bradykinin action in smooth muscle and neurons. J Biol Chem 1995; 270: 13706-13710.

26) Manolis AJ, Marketou ME, Gavras I, Gavras H. Cardioprotective properties of bradykinin: role of the B(2) receptor. Hypertens Res 2010; 33: 772-777.

27) Penna C, Mancardi D, Rastaldo R, Losano G, Pagliaro P. Intermittent activation of bradykinin B2 receptors and mitochondrial KATP channels trigger cardiac postconditioning through redox signaling. Cardiovasc Res 2007; 75: 168-177.

28) Sgarra L, Leo V, Addabbo F, Iacobazzi D, Carratu MR, Montagnani M, Potenza MA. Intermittent losartan administration triggers cardiac post-conditioning in isolated rat hearts: role of BK2 receptors. PLoS One 2014; 9: e88542.

29) Bonde MM, Olsen KB, Erikstrup N, Speerschneider T, Lyngsø C, Haunsø S, Nielsen MS, Sheikh SP, Hansen JL. The angiotensin II type 1 receptor antagonist Losartan binds and activates bradykinin B2 receptor signaling. Regul Pept 2011; 167: 21-25.

30) Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72: 248-254.

31) Tschöpe C, Heringer-Walther S, Walther T. Regulation of the kinin receptors after induction of myocardial infarction: a mini-review. Braz J Med Biol Res 2000; 33: 701-708.

32) Yin H, Chao J, Bader M, Chao L. Differential role of kinin B1 and B2 receptors in ischemia-induced apoptosis and ventricular remodeling. Peptides 2007; 28: 1383-1389.

33) Li HJ, Yin H, Yao YY, Shen B, Bader M, Chao L, Chao J. Tissue kallikrein protects against pressure overload-induced cardiac hypertrophy through kinin B2 receptor and glycogen synthase kinase-3beta activation. Cardiovasc Res 2007; 73: 130-142.

34) Bélanger S, Bovenzi V, Côté J, Neugebauer W, Amblard M, Martinez J, Lammek B, Savard M, Gobeil F Jr. Structure-activity relationships of novel peptide agonists of the human bradykinin B2 receptor. Peptides 2009; 30: 777-787.

35) Toh S, Reichman ME, Houstoun M, Ross Southworth M, Ding X, Hernandez AF, Levenson M, Li L, McCloskey C, Shoaibi A, Wu E, Zornberg G, Hennessy S. Comparative risk for angioedema associated with the use of drugs that target the renin-angioten-

sin-aldosterone system. Arch Intern Med 2012; 172: 1582-1589.

36) Baş M, Greve J, Stelter K, Havel M, Strassen U, Rotter N, Veit J, Schossow B, Hapfelmeier A, Kehl V, Kojda G, Hoffmann TK. A randomized trial of icatibant in ACE-inhibitor-induced angioedema. N Engl J Med 2015; 372: 418-425.

37) Weiss JN, Korge P, Honda HM, Ping P. Role of the mitochondrial permeability transition in myocardial disease. Circ Res 2003; 93: 292-301.

38) Quinlan CL, Costa AD, Costa CL, Pierre SV, Dos Santos P, Garlid KD. Conditioning the heart induces formation of signalosomes that interact with mitochondria to open mitoKATP channels. Am J Physiol Heart Circ Physiol 2008; 295: H953-H961.

39) Savard M, Barbaz D, Belanger S, Müller-Esterl W, Bkaily G, D'orléans-Juste P, Coté J, Bovenzi V, Gobeil F Jr. Expression of endogenous nuclear bradykinin B2 receptors mediating signaling in immediate early gene activation. J Cell Physiol 2008; 216: 234-244.

40) Shah AM, MacCarthy PA. Paracrine and autocrine effects of nitric oxide on myocardial function. Pharmacol Ther 2000; 86: 49-86.

41) Shah AM. Inducible nitric oxide synthase and cardiovascular disease. Cardiovasc Res 2000; 45: 148-155.

42) Sun Y, Carretero OA, Xu J, Rhaleb NE, Wang F, Lin C, Yang JJ, Pagano PJ, Yang XP. Lack of inducible NO synthase reduces oxidative stress and enhances cardiac response to isoproterenol in mice with deoxycorticosterone acetate-salt hypertension. Hypertension 2005; 46: 1355-1361.

43) Yoshida H, Kusama Y, Kodani E, Yasutake M, Takano H, Atarashi H, Kishida H, Takano T. Pharmacological preconditioning with bradykinin affords myocardial protection through NO-dependent mechanisms. Int Heart J 2005; 46: 877-887.

44) Mungrue IN, Gros R, You X, Pirani A, Azad A, Csont T, Schulz R, Butany J, Stewart DJ, Husain M. Cardiomyocyte overexpression of iNOS in mice results in peroxynitrite generation, heart block, and sudden death. J Clin Invest 2002; 109: 735-743.