Bull Yamaguchi Med Sch 58(1-2):7-10, 2011

Bone Marrow Stem Cells Contribute to Cardioprotection in the Late Phase of Ischemic Preconditioning

Takahiro Kamota, Tao-Sheng Li, Noriyasu Morikage, Masanori Murakami, Mako Ohshima, Masayuki Kubo, Toshiro Kobayashi, Akihito Mikamo and Kimikazu Hamano

Department of Surgery and Clinical Science, Division of Cardiac Surgery, Yamaguchi University Graduate School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan
(Received August 29, 2011)

Abstract Ischemic preconditioning (IPC) is a well-known innate phenomenon, in which brief exposure to sublethal ischemia protects organs from subsequent ischemia/reperfusion (I/R) injury. The protective effects after IPC occur in two distinct phases: an early phase and a late phase. However, the exact protective mechanism of IPC is not fully understood, especially in the late phase. Based on increasing evidence that bone marrow stem cells (BMSCs) can repair the injured heart, we investigated whether they also play a role in protecting the heart against I/R injury in the late phase of IPC. We observed an increase in cardioprotective factors in the serum in the early phase, but an increase in BMSCs in the circulating blood in the late phase after remote IPC. Using a heart I/R injury model, we demonstrated that the mobilized BMSCs were recruited into the injured heart and contributed to cardioprotection, especially in the late phase of IPC. Our results indicate that IPC enhances the mobilization and recruitment of BMSCs in the late phase to protect the heart against I/R injury.

Key words: ischemic preconditioning, late phase, bone marrow stem cells, mobilization, recruitment

Introduction

Ischemic preconditioning (IPC), induced by a brief exposure to sublethal ischemia, is an innate protective phenomenon, which markedly reduces ischemia/reperfusion (I/R) injury in various organs.[1] IPC has also been reported to exert systemic effects that protect against I/R injury of tissues remote from those undergoing preconditioning. This is termed "remote IPC".[2] The protective effect after IPC occurs in two distinct phases[3]: the early phase develops rapidly from the time of the initial ischemic insult and lasts for 2 to 3 hours, and the late phase develops 12 to 24 hours after the initial insult and persists for 3 to 4 days (Fig. 1).[4] Until recently, the delayed protective effect of IPC was generally thought to be due to the synthesis of new proteins via the activation of various transcription factors, but expression of some cardioprotective factors has not revealed a second increase peak during the late phase of IPC.[5,6] Interestingly, recent studies have shown the mobilization of bone marrow stem cells (BMSCs) into circulating blood, hours to days after IPC.[7,8] Furthermore, many growth factors released by BMSCs have been found to protect cardiomyocytes from apoptosis,[9] and BMSCs are now used effectively for myocardial repair.[10,11] Thus, we hypothesized that IPC could enhance the mobilization and recruitment of BMSCs, helping to protect the heart against I/R injury in the late phase.

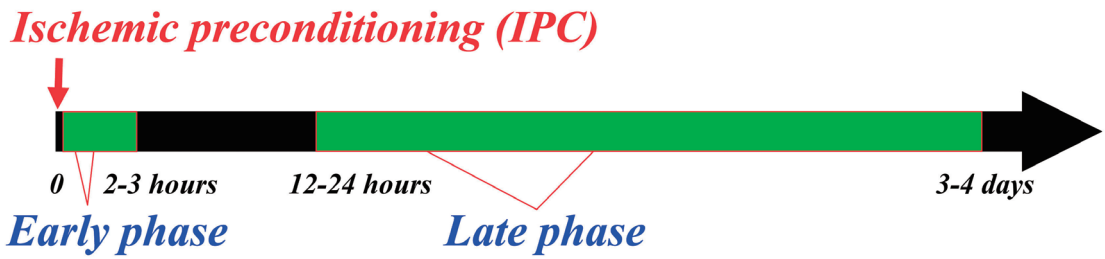

Fig. 1 Two distinct effector phases of IPC for cardioprotection: the early and late phases. The early phase develops rapidly from the time of the initial ischemic insult and lasts for 2 to 3 hours, and the late phase develops 12 to 24 hours after the initial insult and persists for 3 to 4 days.

We were the first to find that IPC-induced mobilization and recruitment of BMSCs plays a critical role in the delayed cardioprotective effects of IPC.[12] Here, we summarize these findings, which were published in detail in the *Journal of the American College of Cardiology*.[12] This paper won the Sojinkai Young Investigator award in 2010.

IPC increased cardioprotective factors in the early phase but enhanced mobilization of BMSCs in the late phase

Cardioprotective factors, including stromal cell-derived factor-1 (SDF-1) and vascular endothelial growth factor (VEGF), in plasma increased 1 and 3 hours (the early phase) after IPC.[12] Conversely, the stem cells in peripheral blood were higher from 12 to 72 hours (the late phase) after IPC but not in the early phase.[12] The phase-specific kinetics of cardioprotective factors and the stem cells indicated that the up-regulation of various cardioprotective factors would contribute to cardioprotection in the early phase of IPC, but the delayed cardioprotection in the late phase of IPC might be related to the mobilization and recruitment of stem cells.

Increased recruitment of BMSCs to the I/R injured heart in the late phase of IPC

In mouse models, we found that IPC could protect the heart against I/R injury in both the early and late phases.[12] The recruitment of BMSCs within the I/R injured hearts was about two-fold higher after the induction of I/R injury in the late phase than in the early phase of IPC.[12] This indicated that the induction of I/R heart injury at different timings of IPC would result in different grades of recruitment of BMSCs. Given the enhanced mobilization of BMSCs in the peripheral blood in the late phase of IPC,[12] it was reasonable to increase the recruitment of BMSCs in the hearts subjected to I/R injury in the late phase of IPC.

Cardioprotective effect was attenuated by blocking the recruitment of BMSCs in the late phase but not in the early phase of IPC

To confirm a relationship between the enhanced recruitment of BMSCs and cardioprotection of late phase IPC, we studied the intervention by blocking the recruitment of BMSCs into the I/R injured heart after IPC.[12] Inhibiting the recruitment of BMSCs by antibody disruption of the SDF-1/CXCR4 axis eliminated almost completely the cardioprotective effects of IPC in the late phase, but not significantly in the early phase.[12] This strongly suggests that the recruitment of BMSCs into the injured heart contributed to cardioprotection in the late phase but not in the early phase of IPC.

The mechanisms of BMSCs for cardioprotection in the late phase of IPC

Previous studies have shown that BMSCs can protect and repair an injured heart by inducing angiogenesis,[10,11,13] differentiating into endothelial cells and myocytes to regenerate new vessel and myocardium,[13] as well as other paracrine mechanisms.[9,13] Although we observed that BMSCs differentiated into endothelial cells and myocytes,[12] the differentiation of BMSCs into endothelial cells is relatively rare and slow, and the differ-

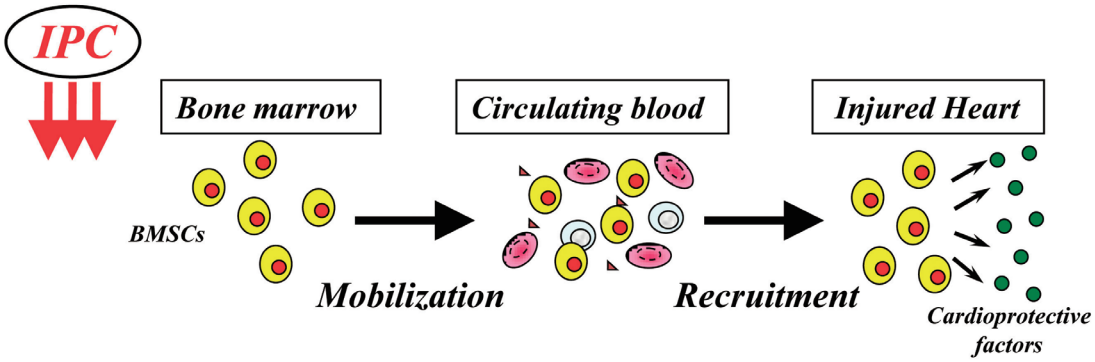

Fig. 2 The cardioprotective mechanisms in the late phase of IPC.
IPC induces the mobilization of BMSCs, and these mobilized BMSCs are recruited to the injured heart; thereby helping to protect the heart against I/R injury through paracrine mechanisms.

entiation of BMSCs into cardiomyocytes is still controversial.[14] Furthermore, BMSCs protected cardiomyocytes against apoptosis through paracrine mechanisms in our co-culture examination.[12] Taken together, it is more likely that the mobilization and recruitment of BMSCs induced by IPC protect the heart against I/R injury by producing various cardioprotective factors, including VEGF, platelet-derived growth factor, and insulin-like growth factor-1,[9,13] rather than by direct repair through the differentiation and maturation of BMSCs.

Conclusions

Our findings indicate that IPC induces the mobilization of BMSCs in the late phase, and that these mobilized BMSCs are recruited to the injured heart; thereby helping to protect the heart against I/R injury through paracrine mechanisms (Fig. 2). This study provides new insight into the cardioprotective mechanisms in the late phase of IPC.

Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture.

Conflict of Interest

The authors state no conflict of interest.

References

1. Murry, C.E., Jennings, R.B. and Reimer, K.A.: Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation,* **74**: 1124-1136, 1986.
2. Przyklenk, K., Bauer, B., Ovize, M., Kloner, R.A. and Whittaker, P.: Regional ischemic preconditioning protects remote virgin myocardium from subsequent sustained coronary occlusion. *Circulation,* **87**: 893-899, 1993.
3. Kuzuya, T., Hoshida, S., Yamashita, N., Fuji, H., Oe, H., Hori, M., Kamada, T. and Tada, M.: Delayed effects of sublethal ischemia on the acquisition of tolerance to ischemia. *Circ. Res.,* **72**: 1293-1299, 1993.
4. Tang, X.L., Qiu, Y., Park, S.W., Sun, J.Z., Kalya, A. and Bolli, R.: Time course of late preconditioning against myocardial stunning in conscious pigs. *Circ. Res.,* **79**: 424-434, 1996.
5. Cai, Z., Manalo, D.J., Wei, G., Rodriguez, E.R., Fox-Talbot, K., Lu, H., Zweier, J. L. and Semenza, G.L.: Hearts from rodents exposed to intermittent hypoxia or erythropoietin are protected against ischemia-reperfusion injury. *Circulation,* **108**: 79-85, 2003.
6. Kawata, H., Yoshida, K., Kawamoto, A., Kurioka, H., Takase, E., Sasaki, Y., Hatanaka, K., Kobayashi, M., Ueyama, T., Hashimoto, T. and Dohi, K.: Ischemic preconditioning upregulates vascular

endothelial growth factor mRNA expression and neovascularization via nuclear translocation of protein kinase C epsilon in the rat ischemic myocardium. *Circ. Res.,* **88**: 696-704, 2001.
7. Ii, M., Nishimura, H., Iwakura, A., Wecker, A., Eaton, E., Asahara, T. and Losordo, D.W.: Endothelial Progenitor cells are rapidly recruited to myocardium and mediate protective effect of ischemic preconditioning via imported nitric oxide synthase activity. *Circulation,* **111**: 1114-1120, 2005.
8. Patschan, D., Krupincza, K., Patschan, S., Zhang, Z., Hamby, C. and Goligorsky, M.S.: Dynamics of mobilization and homing of endothelial progenitor cells after acute renal ischemia: modulation by ischemic preconditioning. *Am. J. Physiol. Renal Physiol.,* **291**: 176-185, 2006.
9. Takahashi, M., Li, T.S., Suzuki, R., Kobayashi, T., Ito, H., Ikeda, Y., Matsuzaki, M. and Hamano, K.: Cytokines produced by bone marrow cells can contribute to functional improvement of the infracted heart by protecting cardiomyocytes from ischemic injury. *Am. J. Physiol. Heart Circ. Physiol.,* **291**: H886-H893, 2006.
10. Strauer, B.E., Brehm, M., Zeus, T., Bartsch, T., Schannwell, C., Antke, C., Sorg, R.V., Kögler, G., Wernet, P., Müller, H.W. and Köstering, M.: Regeneration of human infarcted heart muscle by intracoronary autologous bone marrow cell transplantation in chronic coronary artery disease: the IACT Study. *J. Am. Coll. Cardiol.,* **46**: 1651-1658, 2005.
11. Chen, S.L., Fang, W.W., Ye, F., Liu, Y.H., Qian, J., Shan, S.J., Zhang, J.J., Chunhua, R.Z., Liao, L.M., Lin, S. and Sun, J.P.: Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. *Am. J. Cardiol.,* **94**: 92-95, 2004.
12. Kamota, T., Li, T.S., Morikage, N., Murakami, M., Ohshima, M., Kubo, M., Kobayashi, T., Mikamo, A., Ikeda, Y., Matsuzaki, M. and Hamano, K.: Ischemic preconditioning enhances the mobilization and recruitment of bone marrow stem cells to protect against ischemia/reperfusion injury in the late phase. *J. Am. Coll. Cardiol.,* **53**: 1814-1822, 2009.
13. Kinnaird, T., Stabile, E., Burnett, M.S., Lee, C.W., Barr, S., Fuchs, S. and Epstein, S.E.: Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. *Circ. Res.,* **94**: 678-685, 2004.
14. Alvarez-Dolado, M., Pardal, R., Garcia-Verdugo, J.M., Fike, J.R., Lee, H.O., Pfeffer, K., Lois, C., Morrison, S.J. and Alvarez-Buylla, A.: Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. *Nature,* **425**: 968-973, 2003.