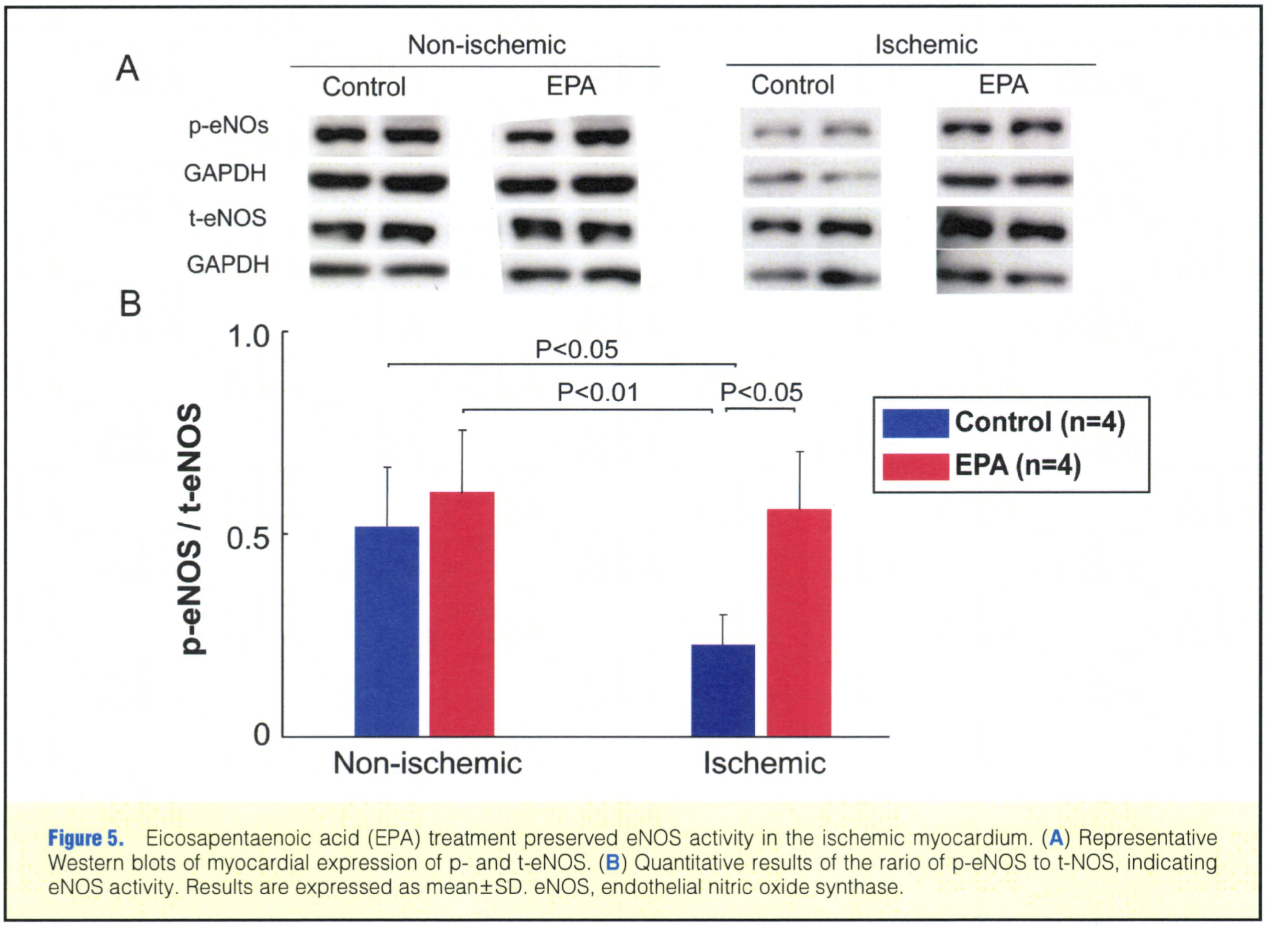

Figure 5. Eicosapentaenoic acid (EPA) treatment preserved eNOS activity in the ischemic myocardium. (**A**) Representative Western blots of myocardial expression of p- and t-eNOS. (**B**) Quantitative results of the rario of p-eNOS to t-NOS, indicating eNOS activity. Results are expressed as mean±SD. eNOS, endothelial nitric oxide synthase.

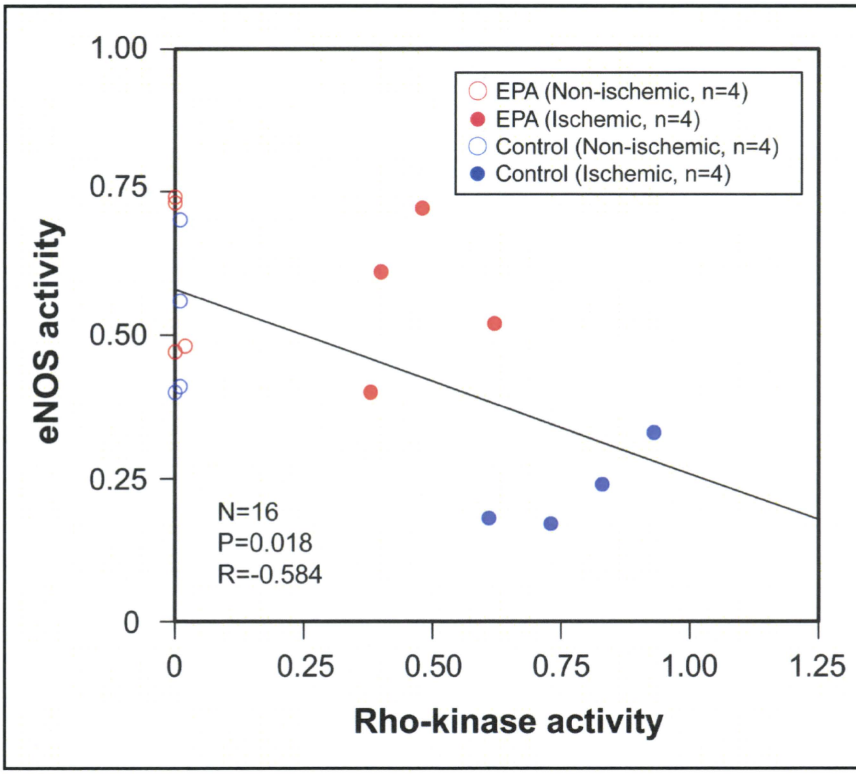

Figure 6. Significant negative correlation between Rho-kinase activity and eNOS activity in the myocardium. A significant negative correlation was noted between Rho-kinase activity and eNOS activity in the myocardium. EPA, eicosapentaenoic acid; eNOS, endothelial nitric oxide synthase.

small GTPase Rho and mediates diverse cellular functions, such as smooth muscle cell contraction, cell migration and proliferation.[15] In a previous study, it has been reported that hydroxyfasudil, a specific Rho-kinase inhibitor, could reduce myocardial infarct size after I/R.[19]

It has been reported that EPA could inhibit sphingosylphosphorylcholine-induced Rho-kinase activation in vitro.[12] However, to the best of our knowledge, the present study is the first study demonstrating that long-term EPA treatment significantly inhibits Rho-kinase activation in the myocardium subjected to I/R in vivo. The Rho-kinase pathway is activated by inflammatory stimuli,[46] which is likely to be involved in neutrophil accumulation after I/R. Platelet activation with a resultant microthrombus formation might also be associated with Rho-kinase pathway activation by releasing serotonin and platelet-derived growth factors and by interaction with thrombin.[47] It also has been reported that expression and activity of Rho-kinase are enhanced by hypoxia.[18]

The present study also demonstrated that the inhibitory effects of EPA on Rho-kinase activation is accompanied with preserved eNOS activity and that there was a negative correlation between Rho-kinase activity and eNOS activity in the myocardium (**Figure 6**). Rho-kinase is involved in the regulation of eNOS activity,[48] where activated Rho-kinase reduces eNOS activity through inhibition of protein kinase B/Akt.[49] Conversely, Rho-kinase inhibition leads to a rapid phosphorylation and activation of Akt via PI3-kinase, leading to increased NO production.[20,50]

There seems to be a discrepancy between Rho-kinase activity (assessed by the extent of the myosin-binding subunit phosphorylation) and its expression in response to I/R injury and EPA treatment. The Rho-kinase was activated following I/R, which was significantly decreased by the EPA treatment, whereas the expression of ROCK-I and -II were not affected by I/R or the treatment. As reported previously, these findings might be related, in part, to the time-course of the myosin-binding subunit –ROCK interaction or cleavage of ROCK.[51,52] Further studies are needed to determine the exact mechanism.

Study Limitations

Several limitations should be mentioned for the present study. First, the present study was designed by using a single dose and performed in normal juvenile pigs without pre-existing atherosclerotic coronary plaques or myocardial dysfunction, both of which could affect myocardial responses to ischemia and reperfusion. Second, because of the potential confounding effect of the relatively short (60 min) reperfusion period, more extended follow up is required to evaluate the effect of infarct size reduction in future studies. Finally, despite the comparable area at risk between the 2 groups, we did not directly measure the area of necrosis following a 90 min-LCX occlusion, which could develop infarction in 30–40% of the area at risk, which was examined by triphenyl tetrazolium chloride staining.[53,54]

Conclusion

In conclusion, the present study demonstrates that long-term treatment with EPA ameliorates myocardial I/R injury partly through the Rho-kinase pathway inhibition in pigs in vivo.

Acknowledgments

The present work was supported, in part, by grants-in-aid [18890018] from the Scientific Research and the global COE project [F02], and grants-in-aid [H22-Shinkin-004] from the Japanese Ministry of Education, Culture, Sports, Science, and Technology, Tokyo, Japan.

The authors wish to thank Dr Masaaki Soma (Mochida Pharmaceutical Co, Ltd) and Dr Takatoshi Hizume (Kyushu University Graduate School of Medicine) for their valuable cooperation in the present study, Mochida Pharmaceutical Co, Ltd for providing EPA and Fumie Tatebayashi and Naomi Yamaki for their excellent technical assistance.

Disclosure

Conflict of interest: none.

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Supplemental Files

Supplemental Files 1

Figure S1. Eicosapentaenoic acid (EPA) treatment attenuates neutrophil infiltration in the ischemic region following ischemia-reperfusion.

Figure S2. Eicosapentaenoic acid (EPA) treatment did not alter the ROCK-I expression in the non-ischemic and the ischemic region.

Figure S3. Eicosapentaenoic acid (EPA) treatment did not alter the myocardial ROCK-II expression, which was decreased in the ischemic region.

Please find supplemental file(s);
http://dx.doi.org/10.1253/circj.CJ-11-0209