Cardioprotective Role of Hepatic Cells in Myocardial Ischemia

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Introduction

Adult cardiomyocytes possess a limited capacity of protection against ischemic injury. Nonmyocytic cells can be activated to support myocardial survivability in myocardial ischemia (MI) (1). Hepatic cells, including hepatocytes and biliary epithelial cells, represent such cell types (2). Hepatocytes can upregulate genes encoding secretory proteins, including AGP2, BMPER, FGF21, NRG4, and TFF3, and can be mobilized to the circulatory system in response to MI (2). Here, we intended to test in a mouse model: (a) whether mobilized hepatic cells were able to engraft to the lesion of ischemic myocardium; (b) whether hepatic cells exerted a beneficial effect on myocardial protection; and (c) whether AGP2, BMPER, FGF21, NRG4, and TFF3 contribute to myocardial protection.

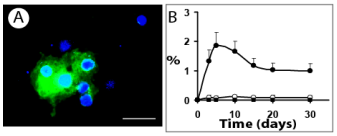
Materials and Methods

Myocardial ischemia was induced by ligating the LAD coronary artery in transgenic Cre-EYFP mice with hepatic cell-specific EYFP expression for cell identification. Myocardial infarction was assessed by TTC and AZAN assays. Hepatic cell mobilization was detected by fluorescence microscopy and flow cytometry. Hepatocyte expression and the serum level of AGP2, BMPER, FGF21, NRG4, and TFF3 were tested by immunoblotting and ELISA, respectively. Recombinant AGP2, BMPER, FGF21, NRG4, and TFF3 were administered IV to evaluate their role in myocardial protection. Partial hepatectomy (~60%) was induced to reduce liver cell mobilization and secretion of AGP2, BMPER, FGF21, NRG4, and TFF3 and thus to test the effect of these processes on myocardial protection.

Results

(a) Hepatic cell mobilization

Hepatic cells genetically marked with EYFP were found in the circulation of mice with MI (Fig. 1 & 2) as well as the lesion of ischemic myocardium (Fig. 3). These cells were not present in the circulation and myocardium of sham-operated mice.



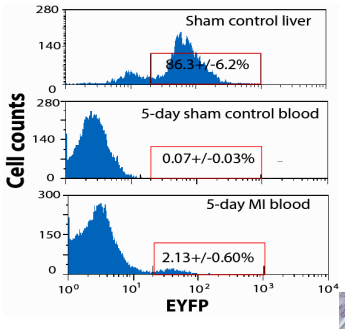


Fig. 1 (left). (A) Circulating EYFP+ cells (green) in 5-day MI. Blue: nuclei. Bar: 10 μm. (B) Relative populations of circulating EYFP+ cells with reference to the total nucleated blood cells in Cre-EYFP mice with sham-operation (open circles) and MI (solid circles) as well as in C57BL/6 mice with MI (solid squares) measured by fluorescence microscopy. $p < 0.001$ for changes in Cre-EYFP mice with MI by ANOVA. n = 6 at each time.

Fig. 2 (left). Cytometry analyses of EYFP+ cells from the liver and circulatory system of Cre-EYFP mice with sham operation and MI. The fraction shown in each panel represents the mean and SD of the EYFP+ cell population from 6 tests.

(b) Role of hepatic cells in myocardial protection

To demonstrate the cardioprotective role of hepatic cells, partial hepatectomy (~60%) was induced to reduce hepatic cell mobilization. A shown in Fig. 4, partial hepatectomy resulted in a significant increase in myocardial infarction. Transplantation of 5-day myocardial ischemia-conditioned hepatic cells (~10^5 cells, 1 IV injection following LAD ligation) reversed changes due to partial hepatectomy (Fig. 4D).

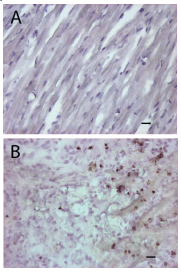


Fig. 3. Horseradish peroxidase-based immunoenzymatic micrographs showing intact myocardium (A) and EYFP+ cells (brown) engrafted to the ischemic myocardium of a Cre-EYFP mouse at day 5. Bar: 10 μm.

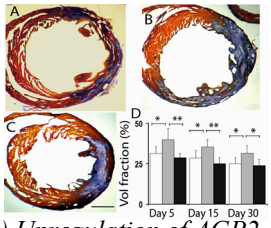


Fig. 4. (A-C) AZAN-stained specimens showing myocardial infarcts (blue) in mice with MI + sham liver operation (A), MI + partial hepatectomy (B), and MI + partial hepatectomy + hepatic cell transplantation (C) at day 5. Bars: 1 mm. (D) Effect of partial hepatectomy on myocardial infarction without (gray) and with (black) hepatic cell transplantation. White: MI with sham liver operation. * $p<0.05$, ** $p<0.01$. n=6 at each time.

(c) Upregulation of AGP2, BMPER, FGF21, NRG4, and TFF3

Myocardial ischemia induced an increase in hepatocyte expression (Fig. 5) and the serum level (Fig. 6) of AGP2, BMPER, FGF21, NRG4, and TFF3. These factors were also increased in sham control mice, but the changes were significantly lower than those in MI at selected times (Fig. 6).

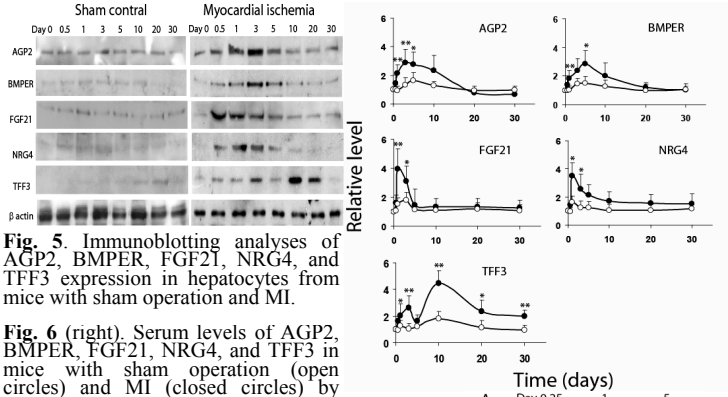


Fig. 5. Immunoblotting analyses of AGP2, BMPER, FGF21, NRG4, and TFF3 expression in hepatocytes from mice with sham operation and MI.

Fig. 6 (right). Serum levels of AGP2, BMPER, FGF21, NRG4, and TFF3 in mice with sham operation (open circles) and MI (closed circles) by ELISA. n = 6 at each time. * $p<0.05$ and ** $p<0.01$.

(d) Cardioprotective role of AGP2, BMPER, FGF21, NRG4, and TFF3

To demonstrate the cardioprotective role of AGP2, BMPER, FGF21, NRG4, and TFF3, partial hepatectomy (~60%) was induced to reduce the secretion of these factors (data not shown). As shown in Fig. 7, partial hepatectomy resulted in a significant increase in myocardial infarction at day 5. Administration of combined AGP2, BMPER, FGF21, NRG4, and TFF3 (25ng/gm, IV injection, twice per day for a max of 3 days starting immediately after LAD ligation) reversed the changes due to partial hepatectomy (Fig. 7B).

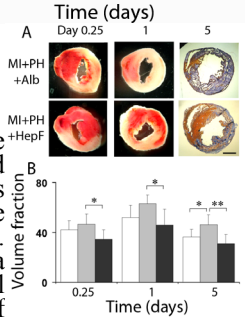


Fig. 7. (A) Myocardial specimens stained with TTC in 0.25- and 1-day MI, and with AZAN in 5-day MI. PH: Partial hepatectomy. Alb: Albumin administration. HepF: Hepatocyte secretory factors AGP2, BMPER, FGF21, NRG4, and TFF3. Bar: 1 mm. (B) Effect of partial hepatectomy on myocardial infarction without (gray) and with (black) administration of combined AGP2, BMPER, FGF21, NRG4, and TFF3. White: MI with sham liver operation. * $p<0.05$, ** $p<0.01$.

Discussion and Conclusions

The present observations suggested that hepatic cells were mobilized in response to myocardial ischemia and exerted a potentially beneficial effect on myocardial survivability. The secretory factors AGP2, BMPER, FGF21, NRG4, and TFF3 were upregulated in hepatocytes and possibly mediated the cardioprotective role of hepatic cells.

References

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