Paracrine cardioprotective signaling by red blood cells through hypoxia-mediated activation of soluble guanylate cyclase and release of cyclic guanosine monophosphate

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Background: Red blood cells (RBCs) are known to regulate cardiovascular function under hypoxic conditions and to mediate cardioprotection via nitric oxide (NO)-like bioactivity. However, the molecular signalling behind this effect and the identity of any mediator released by the RBCs remain unknown. Previous studies revealed that NO activates soluble guanylate cyclase (sGC) and increases formation of the second messenger cyclic guanosine monophosphate (cGMP) in RBCs. The functional role of this signalling in RBCs during hypoxia/ischemia is still unclear.

Aim: To determine the functional role of the NO-sGC-cGMP signalling pathway in RBCs during hypoxia and myocardial ischemia.

Method: RBCs collected from wild-type and sGC knockout mice were exposed to 1% hypoxia or normoxia for 1 h. The RBCs or the extracellular supernatant were then administered to isolated Langendorff-perfused mouse hearts subjected to 40 min global ischemia and 60 min reperfusion. Afterwards, left ventricular developed pressure and infarct size were determined. Additional groups of mice and patients with mild hypertension were given oral nitrate (10mM in their drinking water) before the collection of RBCs to further increase NO signalling.

Result: Administration of RBCs or the extracellular supernatant collected from wild-type mouse RBCs exposed to hypoxia to isolated hearts subjected to ischemia-reperfusion improved post-ischemic cardiac function and reduced infarct size (Fig. 1A and B). By contrast, supernatant collected from hypoxic RBCs of sGC knockout mice failed to induce cardioprotection (Fig. 1C). The cardioprotection induced by hypoxic RBCs of wild-type mice was blocked by MK571, an inhibitor of cyclic nucleotide transport (Fig. 1D). Hypoxia increased extracellular export of cGMP from mouse RBCs (Fig. 1E), and exogenous cGMP resulted in similar cardioprotection induced by the supernatant. The cardioprotective effect of RBCs was blocked by an inhibitor of cardiac cGMP-dependent protein kinase G (PKG) (Fig. 1F) and was associated with increased cardiac PKG-dependent phosphorylation of vasodilator-stimulated phosphoprotein. Oral administration of nitrate to mice for 4 weeks to increase NO bioactivity further enhanced the cardioprotective effect of hypoxic RBCs. RBCs collected from patients randomized to a 5-week nitrate-rich diet induced cardioprotection in the isolated rat heart via an effect dependent on sGC activation in the RBCs (Fig 2).

Conclusion: RBCs generate and export cGMP as a physiological response to hypoxia mediating cardioprotection via a paracrine effect. This effect can be further augmented by a simple dietary intervention with nitrate suggesting preventive and therapeutic opportunities in ischemic heart disease.
Fig. 1. Hypoxia-induced release of cardioprotective cyclic monophosphate (cGMP) from red blood cells (RBCs). (A) Percentage recovery of left ventricular developed pressure (LVDP) during reperfusion and (B) infarct size following administration of supernatant from normoxic and hypoxic RBCs. (C) cGMP in the supernatant of RBCs exposed to normoxia and 1 h of hypoxia. The cardioprotective effect was absent when using RBCs from sGC knockout mice (C) after inhibition of cGMP transport with MK571 (D), associated with the release of cGMP from the RBCs (E), and blocked by the protein kinase G inhibitor KT5823 (F). Data are mean ± SD (n=5–8). ***P<0.001 by two-way ANOVA or unpaired t-test.

Figure 1

Fig. 2. Red blood cells (RBCs) from patients given dietary nitrate mediate cardioprotection. RBCs collected from two groups of subjects randomized to high nitrate intake in the form of nitrate pills or nitrate-rich vegetables and one group subjected to low dietary intake of nitrate were given to isolated rat hearts subjected to ischemia-reperfusion. The cardioprotective effect of RBCs was investigated (A) at baseline before the start of treatment, (B) at follow-up after 5 weeks of treatment, and (C) at follow-up following incubation of the RBCs with the sGC inhibitor ODQ. Data are mean ± SD (n=16). ***P<0.001 by two-way ANOVA.

Figure 2