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Original Contribution

Different target specificities of haptoglobin and hemopexin define a sequential protection system against vascular hemoglobin toxicity

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ABSTRACT

Free hemoglobin (Hb) triggered vascular damage occurs in many hemolytic diseases, such as sickle cell disease, with an unmet need for specific therapeutic interventions. Based on clinical observations the Hb and heme scavenger proteins haptoglobin (Hp) and hemopexin (Hx) have been characterized as a sequential defense system with Hp as the primary protector and Hx as a backup when all Hp is depleted during more severe intravascular hemolysis. In this study we present a mechanistic rationale for this paradigm based on a combined biochemical and cell biological approach directed at understanding the unique roles of Hp and Hx in Hb detoxification. Using a novel in vitro model of Hb triggered endothelial damage, which recapitulates the well-characterized pathophysiologic sequence of oxyHb(Fe^{2+}) transformation to ferric Hb(Fe^{3+}), free heme transfer from ferric Hb(Fe^{3+}) to lipoprotein and subsequent oxidative reactions in the lipophilic phase. The accumulation of toxic lipid peroxidation products liberated during oxidation reactions ultimately lead to endothelial damage characterized by a specific gene expression pattern with reduced cellular ATP and monolayer disintegration. Quantitative analysis of key chemical and biological parameters allowed us to precisely define the mechanisms and concentrations required for Hp and Hx to prevent this toxicity. In the case of Hp we defined an exponential relationship between Hp availability relative to oxyHb(Fe^{2+}) and related protective activity. This exponential relationship demonstrates that large Hp quantities are required to prevent Hb toxicity. In contrast, the linear relationship between Hx concentration and protection defines a highly efficient backup scavenger system during conditions of large excess of free oxyHb(Fe^{2+}) that occurs when all Hp is consumed. The diverse protective function of Hp and Hx in this model can be explained by the different target specificities of the two proteins.

1. Introduction

Hemoglobin (Hb) release from red blood cells (RBC) is a common pathophysiology of many disorders. Systemic hemolysis occurs during certain genetic and acquired anemia, such as in sickle cell disease and malaria. Localized Hb release occurs at confined sites of tissue injury, inflammation, or within atherosclerotic plaques. Depending on the scale, rate, and site of hemolysis, the primary adverse effects triggered by free Hb are vascular dysfunction, oxidative tissue damage, and altered inflammatory response [1–6].

The Hb and heme scavenger proteins haptoglobin (Hp) and hemopexin (Hx) exhibit high binding rate constants for Hb [7] and heme [8], respectively. Both in vitro and in vivo studies have demonstrated the ability of these proteins to attenuate Hb and heme-induced toxicity [9–11]. Under physiologic conditions Hp and Hx exist at comparable plasma concentrations and both are depleted from plasma after binding Hb or heme, respectively. However, in patients with hemolysis Hp depletes early in the course of the disease while levels of Hx remain within the physiologic range for prolonged periods of sustained hemolytic disease [12]. Based on these observations, the two proteins have been characterized as constituents of a sequential Hb/heme protection system with Hp as the primary defense and Hx as the backup, which provides protection against more severe Hb exposure once Hp becomes depleted [12,13]. The biochemical basis of this paradigm of sequential protection by Hp and Hx has yet to be explored systematically.

The vasculature is one of the principal targets of Hb toxicity [14]. Many studies have explored basic mechanisms of Hb or heme

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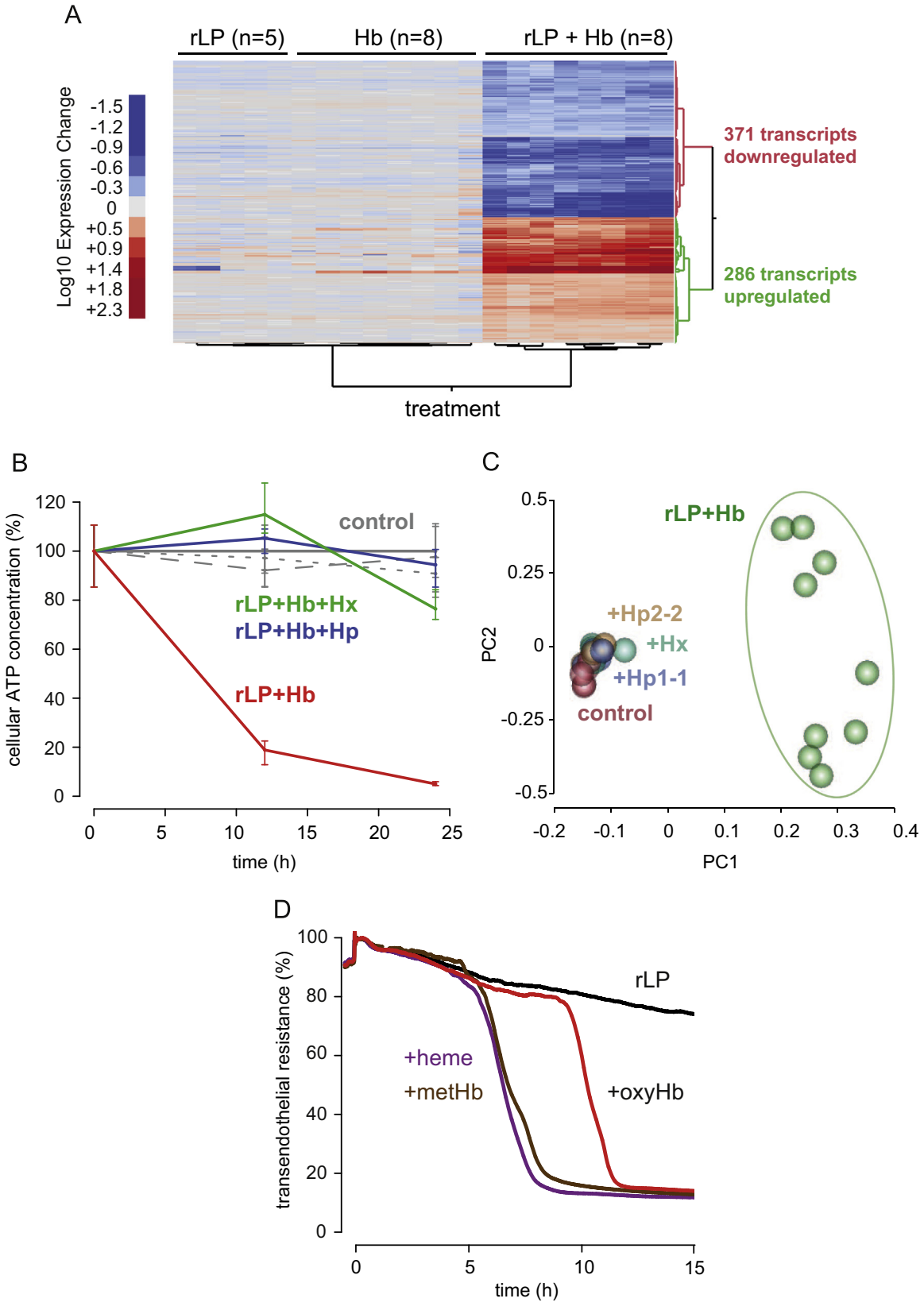

Fig. 1. Effects of combined treatment of rLP and Hb on human umbilical vein endothelial cells. A. Hierarchical two-way clustering analysis of whole genome expression arrays. HUVEC where treated for 9 h with rLP (0.5 mg/ml), Hb (10 μM), or both. Only genes found to exhibit statistically significant differential regulation by ANOVA are shown. The clustering indicates clear separation of the treatment conditions with most pronounced gene expression changes for combined rLP+Hb treatment. Details of gene expression data can be found in the data supplement. B. Time course of cellular ATP depletion. The rLP (0.5 mg/ml)+Hb (10 μM) triggered damage response in HUVEC cells is prevented by Hp and Hx added at excess concentration over Hb (each at 15 μM heme-equivalent binding capacity). C. Principal component analysis of gene expression profiles of untreated HUVEC or HUVEC treated with rLP+Hb in the absence of any scavenger or with Hx or Hp of dimeric or multi-meric phenotype (Hp 1–1 and Hp 2–2). The data confirm that the Hb+rLP triggered gene expression changes are prevented by Hx and Hp. The axes indicate principal components (PC1 and PC2). D. Electric cell-substrate impedance sensing (ECIS) of HUVEC treated with rLP alone or in combination with heme, metHb (Fe3+), or oxyHb (Fe2+). rLP in combination with oxyHb leads to disruption of endothelial monolayer integrity after about 10 h. If oxyHb is replaced by ferric Hb or free heme, the time to disruption is shortened to about 6 h. Single treatment with heme, oxyHb or ferric Hb does not change monolayer integrity (not shown).