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Interaction of recombinant octameric hemoglobin with endothelial cells

Interaction de l'hémoglobine octamérique recombinante avec les cellules endothéliales

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ABSTRACT

Hemoglobin-based oxygen carriers (HBOCs) may generate oxidative stress, vasoconstriction and inflammation. To reduce these undesirable vasoactive properties, we increased hemoglobin (Hb) molecular size by genetic engineering with octameric Hb, recombinant (r) HbβG83C. We investigate the potential side effects of rHbβG83C on endothelial cells. The rHbβG83C has no impact on cell viability, and induces a huge repression of endothelial nitric oxide synthase gene transcription, a marker of vasomotion. No induction of Intermolecular-Adhesion Molecule 1 and E-selectin (inflammatory markers) transcription was seen. In the presence of rHbβG83C, the transcription of heme oxygenase-1 (oxidative stress marker) is weakly increased compared to the two other HBOCs (references) or Voluven (control). This genetically engineered octameric Hb, based on a human Hb βG83C mutant, leads to little impact at the level of endothelial cell inflammatory response and thus appears as an interesting molecule for HBOC development.

Mots clés :
Molécules d'adhésion
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RÉSUMÉ

Les transporteurs d'oxygène à base d'hémoglobine (HBOCs) peuvent induire stress oxydant, vasoconstriction et inflammation. Afin de réduire ces propriétés vasoactives indésirables, nous avons augmenté, par génie génétique, la taille moléculaire de l'hémoglobine (Hb) produisant une Hb octamérique recombinante (r), la rHbβG83C. La rHbβG83C n'a pas d'impact sur la viabilité des cellules endothéliales et induit une répression très importante de la transcription du gène de la NO synthase (marqueur de

Abbreviations: HBOCs, hemoglobin-based oxygen carriers; Dex-BTC-Hb, human Hb conjugated to dextran benzene tetracarboxylate macromolecules; eNOS, endothelial nitric oxide synthase; ICAM-1, intermolecular-adhesion molecule 1; HUVEC, human umbilical vein endothelial cells; PS, phosphatidylserine; PI, propidium iodide; HO, heme oxygenase.

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vasoactivité). Aucune induction de la transcription de gènes des molécules d'adhésion ICAM-1 et E-sélectine (marqueurs d'inflammation) n'a été mise en évidence. En présence de rHbβG83C, la transcription du gène de l'hème oxygénase-I (marqueur de stress oxydant) est faiblement augmentée en comparaison du cas des deux autres HBOCs références et au Voluven (témoin). La rHbβG83C, basée sur un mutant de l'Hb humaine, présente moins d'impact au niveau de la réponse inflammatoire des cellules endothéliales et semble donc être une molécule intéressante pour le développement d'un HBOC.

1. Introduction

The development of hemoglobin (Hb) based oxygen carriers (HBOC) as a blood substitute remains a challenge. However, specific problems are encountered with HBOCs due to the use of acellular Hb: the oxygen affinity, the dimerization and the irreversible auto-oxidation of the Hb [1]. In the past years, the regulation of oxygen affinity has been very well investigated and different studies have shown that it is possible to modulate the oxygen affinity of Hb [2–5]. In parallel, different molecules have been developed to prevent tetramer dissociation and renal toxicity either by chemical modification [6] or by protein engineering technology [1] but clinical trials with stabilized tetrameric Hb have shown an arterial hypertension and peripheral vasoconstriction [7]. This vasoconstriction is essentially attributed to scavenging of nitric oxide (NO) by Hb after extravasation through or close contact with the endothelium [8,9]. The increase of the molecular size of the carrier and its oxygen affinity has been proposed to reduce the undesirable vasoactive properties. Different approaches have been developed to increase the size of HBOC, such as surface modification of different Hbs by poly (ethylene) glycol (PEG) conjugation [10] or by glutaraldehyde polymerization [11]. Generally, these different Hb derivatives are a heterogeneous mixture of different polymers with small components, and may overcome the extravasation and vasoactive effects of acellular Hb. A PEG-Hb conjugate (MP4) has been developed with a molecular weight of 90 kDa and exhibiting a high oxygen affinity [12]. For the first generations of HBOC, the objective was to decrease the oxygen affinity in order to deliver quickly the oxygen to the tissue but the recent study with the MP4 molecule has shown that this macromolecule reduces precapillary oxygen release leading to a more efficient oxygen transport and may help limit the vasoconstriction of the arterioles as a regulatory mechanism for excess oxygen delivery [13,14]. In order to obtain a HBOC with appropriate size and correct oxyphoric capacity, we have developed the recombinant HbβG83C (rHbβG83C) which forms a stable octameric rHb of molecular mass 129 kDa (Fig. 1) with a slightly increased oxygen affinity relative to a Hb A solution [15]. We have previously reported that this octameric rHbβG83C is resistant toward potential disulfide reducing agents present in fresh human plasma and does not interact rapidly with haptoglobin, a glycoprotein that binds Hb (αβ) dimers as part of the elimination of Hb from blood circulation [15–18]. In contrast to other molecules produced by recombinant technology [19], rHbβG83 C exhibits a homogeneous size and does not dissociate into small molecular species at low concentration [15].

In this work, we explore the potential side effects of this octameric molecule on endothelial cells. It has been shown that certain HBOCs in contact with endothelial cells lead to oxidative stress, NO scavenging and inflammatory effects [20–22]. The inflammation induces the expression of molecules such as Intermolecular-Adhesion Molecule 1 (ICAM-1) and E-selectin [21,23]. The presence of oxidized Hb and the products of heme degradation as well as reactive oxygen species induce an oxidative stress response of endothelial cells by the expression of heme oxygenase 1 (HO-1) [23]. One of the major consequences of increased vascular reactive oxygen species production is a reduction of endothelial NO bioavailability [24]. This reduction of NO bioavailability is involved in the mechanism of vasoconstriction observed *in vivo* after the administration of HBOC [25]. Both NO synthesis and endothelial nitric oxide synthase (eNOS) transcriptional regulation are of interest as an increase in eNOS transcription could run counter to NO depletion in the circulation [21].

The aim of the present study was to evaluate the potential impacts of purified octameric rHbβG83C solution on Human Umbilical Vein Endothelial Cells (HUVEC) by assessing Hb effects on the markers of vasoactivity and inflammation and oxidative stress. The results were compared with those obtained with two reference HBOCs, Dex-BTC-Hb, a human Hb conjugated to dextran benzene tetracarboxylate (Dex-BTC) macromolecules [26] described with a decreased oxygen affinity and an increased vascular half-life [27], and Oxyglobin a glutaraldehyde-polymerized bovine Hb (Biopure Corporation, USA), as well as with those obtained with the control Voluven, a plasma expander solution.

2. Materials and methods

2.1. Preparation of HBOCs

The rHbβG83C was produced in BLi5 strains of *Escherichia coli* containing the mutated pHE7 vector [17] and solubilized and purified as previously described [15,16]. The pure octameric rHb were then obtained after size exclusion chromatography on column Superose