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Corticosteroid enhances heme oxygenase-1 production by circulating monocytes by up-regulating hemoglobin scavenger receptor and amplifying the receptor-mediated uptake of hemoglobin–haptoglobin complex

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Abstract

This study examined the relationship between steroid treatment and CD163-mediated downstream pathways linked to inflammatory resolution. Twelve patients referred for congenital heart disease surgery were divided into two groups based on the severity of intravascular hemolysis during cardiopulmonary bypass surgery. Patients with severe intravascular hemolysis were administered haptoglobin during the procedure. Flow cytometry indicated a peak in monocyte CD163 expression on post-operative day 1 in both groups. Enhanced and prolonged heme oxygenase-1 (HO-1) mRNA expression levels were observed in patients who received haptoglobin. Binding of hemoglobin–haptoglobin complex (Hb/Hp) to CD163 resulted in significant induction of HO-1 by peripheral blood mononuclear cells after exposure to dexamethasone prior to culture. This effect was significantly inhibited by anti-CD163 antibody. Our results demonstrated up-regulation of CD163 expression on the monocyte surface by steroid treatment. Steroid treatment was suggested to facilitate CD163-mediated endocytosis of hemoglobin to monocytes/macrophages and thereby induce acceleration of HO-1 synthesis.

Keywords: Cardiopulmonary bypass; Heme oxygenase-1; CD163; Hemoglobin–haptoglobin complex; Steroid; Congenital heart disease

Post-operative damage to the cardiac tissue is a major concern during surgery for congenital heart disease (CHD). Myocardial dysfunction after cardiopulmonary bypass (CPB) surgery is primarily due to bypass-related inflammation of the cardiac tissue [1]. Although systemic steroids seem to be effective in reducing the inflammatory reactions and minimizing post-surgical functional derangement of the heart, the precise mechanism of this effect and the optimum method of steroid administration are not yet clear.

Heme is a product of hemoglobin degradation and acts as a potent inducer of inflammation. Excess free heme may constitute a major threat because heme catalyzes the formation of reactive oxygen species, resulting in oxidative stress, leading to subsequent cell injury. Large amounts of free heme proteins are released during hemolysis under pathological conditions, such as CPB surgery. Macrophages play a role as the primary scavengers of hemoproteins under these conditions.

CD163 is expressed on tissue macrophage and is readily induced upon stimulation of circulating monocytes [2]. CD163 expression has been shown to be tightly regulated

Abbreviations: CHD, congenital heart disease; CO, carbon monoxide; CPB, cardiopulmonary bypass; HO-1, heme oxygenase-1; PBMC, peripheral blood mononuclear cell; Hb/Hp, hemoglobin–haptoglobin complex.

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by pro- and anti-inflammatory mediators in monocytes/macrophages *in vitro* [3] Increased expression of CD163 has been observed in macrophages in inflamed tissue under conditions such as atherosclerosis [4]. Activation of macrophages by interferon-γ and tumor necrosis factor leads to the decreased expression of CD163 [5], whereas the expression of CD163 is highly induced by steroids, IL-4, IL-6, and IL-10 [5–7], Moreover, CD163 has been shown to function as a specific receptor for hemoglobin–haptoglobin (Hb/Hp) complex, thereby playing a significant role as a scavenger of hemoglobin-induced tissue injury [8].

Heme oxygenase (HO) is a potent anti-inflammatory and anti-oxidative enzyme, which is involved primarily in the degradation of heme into biliverdin, carbon monoxide (CO), and free iron. HO-1 has been suggested to act as a potent cytoprotective enzyme through the anti-oxidative activity of biliverdin and its metabolite bilirubin, as well as the anti-inflammatory effects of CO [9,10]. Indeed, deficiency of HO-1 in humans leads to a marked increase in circulating heme and subsequent oxidative vascular and tissue injury, anemia, and chronic inflammation [11]. Therefore, it is crucial to eliminate hemoglobin from the circulation and to induce its downstream metabolite pathway to reduce systemic inflammatory responses and promote tissue healing after CPB surgery [12].

In the present study, we examined whether steroid treatment enhances the synthesis of HO-1 by Hb/Hp binding to CD163.

Methods

Subjects. Twelve patients undergoing CPB surgery for CHD between July and December 2004 were included in the study. Preoperative diagnoses were atrial septal defect ($n = 6$) and ventricular septal defect ($n = 6$). Surgery was performed in all patients by one of the authors (S.A.) and all patients received intraoperative methylprednisolone (MP) (30 mg/kg) intravenously before CPB. Patients were divided into 2 groups with or without intraoperative Hp treatment (200–2500 U) during CPB surgery. One group (MP/Hp, $n = 7$) with macroscopic hematuria received intraoperative Hp in addition to MP as severe intravascular hemolysis was suggested. The other group without hematuria (MP, $n = 5$) received intraoperative MP alone. Clinical outcome data of these patients are summarized in Table 1.

Controls were healthy volunteers who had not received systemic steroid at any time during the last 6 months.

The Ethical Committee at the Faculty of Medicine, Kanazawa University, approved the study protocol, and written informed consent was obtained from the guardians of each patient. Patients requiring emergency treatment, reoperation, or with sepsis, acute or chronic lung disease, immunodeficiency, gastrointestinal bleeding, or steroid use within 2 weeks before surgery were excluded from the study.

Blood samples were obtained via an indwelling radial artery catheter before induction of anesthesia (pre-CPB), 30 min after completion of surgery (end-surgery), 3 and 6 h after surgery, and on post-operative days 1 (18 h) and 2 (42 h). Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples as described previously [13]. Plasma and cell lysates were collected and stored at −70 °C until analysis.

Enzyme-linked immunosorbent assay (ELISA). Plasma IL-6, IL-10, soluble CD163, and Hb/Hp complex levels were measured by ELISA according to the manufacturer's instructions (IL-6; TECHNE Co., Minneapolis, MN, USA, IL-10; eBioscience, San Diego, CA, USA, soluble CD163; BMA Biomedicals, Augst, Switzerland, and Hb/Hp complex; Immundiagnostik AG, Bensheim, Germany).

FACS analysis. The expression of surface antigens on PBMC, including CD163, HLA-DR, CD16, CD14, and ICAM-1, was analyzed by FACSCalibur flow cytometer (Becton–Dickinson, San Diego, CA, USA) using CellQuest software (Becton–Dickinson) as described previously [14].

Real-time reverse transcription-polymerase chain reaction (real time RT-PCR). HO-1 and CD163 expression in PBMCs were assessed by RT-PCR as described previously [14]. The PCR primers for human HO-1, CD163, and GAPDH were as follows: sense primer 5′-TGAGGAACTTTCAGAAGGGCC-3′, antisense primer 5′-TGTTGCGCTCAATCTCCTCC-3′; probe oligonucleotide 5′-CGGCTTCAAGCTGGTGATGGCC-3′ for HO-1, and sense primer 5′-TTGAAGACTCTGGATCTGCTGAC-3′, and antisense primer 5′-TCCACTAGCCTCAGCTCCTTG-3′ for CD163, and sense primer 5′-GAAGGTGAAGGTCGGAGTC-3′, antisense primer 5′-GAAGATGGTGATGGGATTTC-3′, and probe oligonucleotide 5′-CAAGCTTCCCGTTCTCAGCC-3′ for GAPDH.

PBMC in vitro culture experiments. Human PBMCs were isolated from venous blood samples. Isolated PBMCs were cultured in 6-well plates at 1×10^7 cells/well in RPMI-1640 medium (Gibco Laboratories, Grand

Table 1
Clinical outcome data

	Intraoperative MP + Hp administration	Intraoperative MP only	*P*
Mechanical ventilation (h)	11 ± 2	8 ± 4	0.23
Blood loss (mL)	350 ± 90	256 ± 145	0.40
Transfusion (packed red blood cells, units)	4.7 ± 2.7	1.8 ± 2.0	0.09
Intubation time (h)	10.5 ± 2.5	8.1 ± 4.3	0.23
Inotropic support (h)	17 ± 9	12 ± 11	0.43
Fluid input/output over first 24 h			
Fluid input (L/m^2/day)	1.7 ± 0.6	1.5 ± 0.5	0.52
Fluid output (L/m^2/day)	2.1 ± 0.8	2.4 ± 0.5	0.54
Body temperature first 24 h after CPB (°C)			
Average body temperature	37.3 ± 0.3	37.0 ± 0.5	0.29
Maximum body temperature	38.7 ± 0.5	38.4 ± 0.2	0.10
Estimated creatinine clearance (mL/min/m^2)			
Preoperative	87 ± 16	98 ± 6	0.19
ICU admit	83 ± 10	100 ± 17	0.06
Post-operative day 1	79 ± 30	109 ± 13	0.06
Post-operative day 2	86 ± 14	110 ± 12	0.10

Data are expressed as means ± SD. ICU indicates intensive care unit.