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PEGylated albumin-heme as an oxygen-carrying plasma expander: Exchange transfusion into acute anemia rat model

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Abstract

Poly(ethylene glycol) (PEG) conjugated recombinant human serum albumin (HSA) incorporating the synthetic iron-porphyrin (FeP) [PEGylated albumin-heme, PEG(HSA–FeP)] is a unique albumin-based oxygen carrier as a red blood cell (RBC) substitute. The physiological responses to an exchange transfusion with PEG(HSA–FeP) into an acute anemia rat model were investigated. After a 65% isovolemic hemodilution with HSA, a 30% volume of the circulatory blood was withdrawn, affording a hemorrhaged state. The circulation parameters, blood parameters, renal cortical oxygen partial pressure [PtO_2(R)], and muscle tissue oxygen partial pressure [PtO_2(M)] were continuously monitored. The intravenous infusion of PEG(HSA–FeP) restored the reduced levels of the mean arterial pressure, heart rate, respiration rate, mixed venous PO_2, and arterial PCO_2. The increased arterial PO_2 and pH also returned to their basal values. These effects were almost to the same extent as those observed after the administration of the RBC suspension. The relatively low recovery in PtO_2(R) and PtO_2(M) might be due to the Langmuir-type oxygen binding profile of PEG(HSA–FeP) (Hill coefficient: 1.0). All the animals survived during the experiments. In contrast, those injected with HSA died within 41 min. The PEG(HSA–FeP) solution is an oxygen-carrying plasma expander which can be used as a resuscitative fluid for hemorrhagic shock.

Keywords: Albumin; Biomimetic material; Blood; In vivo test; Oxygenation; Poly(ethylene glycol)

1. Introduction

Human hemoglobin (Hb)-based oxygen carriers as a blood replacement composition have been vigorously developed in the past decade [1–3], e.g., polymerized Hb [4], polymer-conjugated Hb [5], and phospholipid vesicle encapsulated Hb [6,7]. Some of them have already been used in clinical Phase II/III trials. The most superior property of these materials is certainly "no blood type". One can administer these materials into patients who need a blood transfusion without cross matching and typing before use. This saves time and facilities, allowing instant transfusion, which is tremendously useful in an emergency. On the other hand, the largest concern of the Hb-products is the source of the human Hb, which is regulated by the availability of donated human blood.

Based on this background, poly(ethylene glycol) (PEG) conjugated human serum albumin (HSA) incorporating 2-[8-{*N*-(2-methylimidazolyl)}octanoyloxymethyl]-5,10,15,20-tetrakis{α,α,α,α-*o*-(1-methylcyclohexanamido)phenyl}porphinatoiron(II) (FeP, Chart 1) [PEGylated albumin-heme, PEG(HSA–FeP)] has been developed as a unique albumin-based oxygen carrier [8]. Recombinant HSA is now manufactured on an industrial scale (one million vials per year) [9] and the batch production of the synthetic FeP has also been established [10]. The oxygen binding affinity (oxygen pressure where 50% of heme is oxygenated) of PEG(HSA–FeP) (P_{50}) was adjusted to 32 Torr (at 37 °C) that is similar to the 28 Torr of human RBC, and the solution properties are almost the same as those of HSA itself. The surface modification with PEG significantly improved not only the

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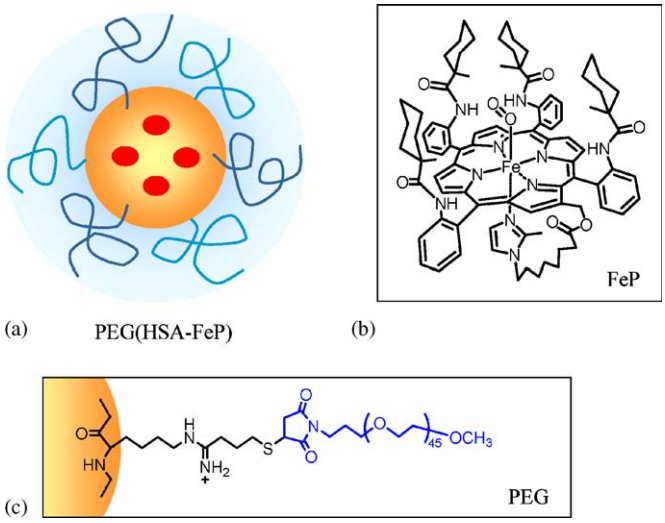

Chart 1. (a) Schematic illustration of PEGylated albumin-heme [PEG(HSA–FeP)], (b) chemical structure of FeP, and (c) binding form of PEG to the lysine group of HSA.

circulation lifetime of FeP in vivo, but also the stability of the oxygenated complex [8]. The PEG(HSA–FeP) solution would be of extreme medical importance as a new type of oxygen-carrying plasma expander.

We now report for the first time the systemic evaluations of the physiological responses to an exchange transfusion with PEG(HSA–FeP) into an acute anemia rat model. The animals were first placed in a 65 vol% hemodilution with HSA and then underwent a 30 vol% blood replacement with PEG(HSA–FeP). The circulation parameters, blood parameters, the oxygen deliveries to the renal cortex and the muscle tissue were monitored and compared to the HSA group and RBC group for 120 min after the infusion.

2. Materials and methods

2.1. Preparation of PEG(HSA–FeP) solution

The 5 g/dL HSA solution was prepared by dilution of recombinant HSA [Albrec®, 25 wt%, NIPRO Corp. (Osaka)] with a saline (Otsuka Pharmaceutical Co., Ltd.). The PEG(HSA–FeP) was prepared according to our previously reported procedure [8] (phosphate buffer saline solution, pH = 7.4, [HSA] = 5 g/dL, [FeP] = 3 mM (FeP/HSA = 4 mol/mol), Mw of PEG = 2333 Da, averaged number of PEG per HSA–FeP = 6, oxygen-binding affinity (P_{50}) = 32 Torr (37 °C), density = 1.01 g/cm^3, colloid osmotic pressure = 27 mmHg, viscosity = 1.14 cP).

2.2. Extreme hemodilution and exchange transfusion

The investigations were carried out with 15 male Wistar rats (288 ± 18 g). The methods of operation were described elsewhere in detail [11].

The animals were under an inhalation anesthesia with sevoflurane; its concentration was kept at 1.5% during the experiment. First, a 65% hemodilution was carried out using 5 g/dL HSA. The blood withdrawal via the common carotid artery (2 mL) and the HSA infusion from the femoral vein (2 mL) (each 1 mL/min) were repeated for eight cycles. After 10 min, a 30% volume of the circulatory blood was withdrawn, and the identical volume of PEG(HSA–FeP) was injected ($n = 5$) (1 mL/min). As negative or positive-control group, the 5 g/dL HSA solution (HSA group, $n = 5$) or the washed RBC suspension (RBC group, $n = 5$) was infused to the similarly operated rats in hemorrhage. The washed RBC suspension was prepared as follows. The fresh withdrawn whole rat blood in the heparinized tube was centrifuged and the plasma layer was discarded. The 5 g/dL HSA was added to the tube and centrifuged again. Then, the supernatant was discarded and 5 g/dL HSA was added to adjust the Hb concentration to 5 g/dL ([heme] = 3 mM).

The blood samples from the artery (0.3 mL) and vein (0.2 mL) were collected at the following seven time-points: (1) before the 65% hemodilution, (2) immediately after the hemodilution, (3) 10 min after the hemodiluation, (4) immediately after the 30% bleeding, (5) immediately after the sample infusion, (6) 60 min, and (7) 120 min after the infusion. Mean arterial pressure (MAP) and heart rate (HR) were recorded by a Polygraph System (NIHON KODEN LEG-1000 Ver. 01-02 or PEG-1000 Ver. 01-01) at the following eleven time-points; (1) before the 65% hemodilution, (2) immediately after the hemodilution, (3) 10 min after the hemodilution, (4) immediately after the 30% bleeding, (5) immediately after the sample infusion, (6) 5 min, (7) 15 min, (8) 30 min, (9) 60 min, (10) 90 min, and (11) 120 min after the sample infusion. Collected blood sample was applied to a blood gas system (Radio Meter Trading ABL555) to measure the oxygen pressure (PaO_2), carbon dioxide pressure ($PaCO_2$) and pH of the arterial blood, and the oxygen pressure (PvO_2) and lactate of the venous blood. Renal cortical oxygen partial pressure [PtO_2(R)] and muscle tissue oxygen partial pressure [PtO_2(M)] were monitored by a tissue oxygen pressure monitor (Inter Medical PO_2-100 DW) using a polarographic oxygen-electrode (Intermedical POE-10 N and POE-40PS) inserted into the left renal cortex and muscle in the abdomen.

The animals were sacrificed after the experiments by venesection. All animal handling and care were in accordance with the NIH guidelines. The protocol details were approved by the Animal Care and Use Committee of Keio University.

2.3. Statistical analysis

All data were represented by mean ± standard deviation (SD). Statistical analyses ware performed using the Tukey–Kramer multiple comparison test for three groups, and by repeated measures analysis of variance followed by paired t-test. The software used was a StatView (SAS Institute, Inc.). Values of $p < 0.05$ were considered significant.