Developmental Trend of Artificial Blood (Artificial Red Blood Cells)

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Abstract: Regarding research on artificial blood, the “Field of Artificial Blood Development” was inaugurated in 1997, supported by the Ministry of Health and Welfare Grant-in-Aid for Health Science Research, for intensive research activities in the three sub-fields, i.e., artificial red blood cells, artificial platelets, and artificial antibodies. Developed by molecular assembling technology, artificial red blood cells, in the form of hemoglobin vesicles comprising hemoglobin encapsulated with a phospholipid bilayer as a highly efficient oxygen carrier, are now under investigation in laboratory animals to verify their function and safety. These vesicles are characterized by a particle size about 1/30 that of erythrocytes, preservability in a liquid state for 2 years at room temperature, and a sufficient retention time in circulating blood without evoking activation of platelet or complements. The hemoglobin vesicles have proven both to possess a high oxygen-carrying capacity in massive exchange transfusion studies in rodents, and to be remarkably safe, based on blood biochemical tests and pathologic findings in load-dosing and repeated-dose studies. Their noticeable safety against active oxygen has also been demonstrated. A joint industry, government, and university research project on artificial red blood cells is in progress with the present objective of developing a complement to transfusion therapy for emergency lifesaving.

Key words: Artificial blood; Artificial red blood cells; Hemoglobin vesicles; Function and safety evaluation

Introduction

We humans and other animals are constantly left exposed to the ferocity of certain viruses, and blood services are substantially affected by those viral entities. In Japan, the “Field of Artificial Blood Development” was inaugurated in 1997 as a Health Science Research—Advanced Frontier Medical Research Project, whereby intensive research activities in the three sub-fields, i.e., artificial red blood cells, artificial platelets, and artificial antibodies, are being pursued. Artificial blood is expected to have a significant influence upon the progress...
of medical care in the 21st century by complementing current blood products for transfusion, and creating a stable supply of safe products. Promotion of the research and development aimed at commercialization of artificial blood has been set as a basic policy of this country (a Resolution at the Health, Labour and Welfare Committee of the House of Representatives, July 24, 2002: a matter concerning promotion of the safety measures for pharmaceuticals and medical devices).

This article will focus upon artificial red blood cells, of which practical application is close to becoming a reality. The following are anticipated from its materialization: (1) feasible blood transfusion without regard to selection of blood group/type in case of an emergency, (2) no need of apprehension of HIV, hepatitis, and other viral or bacterial infections inclusive of unknown viruses, and (3) practicable massive reserves so that accidents in disasters such as earthquakes can be immediately coped with.

Present Status of Artificial Red Blood Cell Development

Materials such as perfluorocarbon emulsion and modified hemoglobin have been assessed and clinically used as artificial red blood cells, but none has proven to be satisfactory from the viewpoints of function and safety. The hemoglobin vesicles (HbV) comprising a high-concentration hemoglobin encapsulated with phospholipid bilayer, hence analogous to erythrocytes, which are currently under investigation in Japan (Fig. 1), are safest and promising for practical use.1,2 While effective utilization of hemoglobin from expired donated blood is being put forward at the present stage, use of recombinant hemoglobin will probably be utilized in the future.

Blood group substances, proteins other than hemoglobin, and viruses (if present at all) are completely removed by heating and filtration through the process of hemoglobin purification from erythrocytes. Re-encapsulation with a stable lipid membrane ensures the preservability of the product in the liquid state for 2 years at room temperature (in contrast to the current erythrocyte preparations which may be stored for 3 weeks with refrigeration after blood drawing). When stored in the form of dry powder, the product can be preserved for a longer period. These are generally thought to be great advantages of the artificial blood product.

The research on the HbV is being pursued as a cooperative study (aided by a Grant-in-Aid for Health-Labour Science Research) mainly by the study group headed by Prof. Emeritus Eishun Tsuchida at the Advanced Research Institute for Science and Engineering, Waseda University, where the author is affiliated, and the study groups headed by Prof. Koichi Kobayashi and Prof. Makoto Suematsu at Keio University School of Medicine. In collaboration with a private enterprise, the project aims at finalization of the pharmaceutical formulation and an early initiation of its clinical trials.

Evaluation of Function and Safety of Hemoglobin Vesicles (HbV)

The physical and chemical properties of the HbV are specified in Table 1. The colloidal osmotic pressure is practically zero because hemoglobin is encapsulated. It will be likened to a state where erythrocytes are dispersed in physiological saline. In the case where the colloidal osmotic pressure is to be adjusted,
The studies conducted to assess the HbV heretofore obtained. The studies were performed mostly in rats and hamsters, and have sufficiently verified the basic safety and oxygen-carrying effect. A safety study in primates is in progress at present.

In rats with 90% of the total blood volume replaced with albumin solution alone to explore the oxygen-carrying effect, decreases in systemic blood pressure and renal cortical oxygen partial pressure became conspicuous after an approximately 70% exchange, resulting in death. When the exchange was carried out using a system consisting of HbV dispersed in albumin solution, both the systemic blood pressure and renal cortical oxygen partial pressure were maintained even after a 90% exchange. In an 80% exchange transfusion study with HbV dispersions in albumin solution conducted in hamsters, tissue oxygen partial pressure in the subcutaneous microcirculation system, as measured non-invasively, was noted to have decreased to 60–70% of pre-exchange value, yet it was maintained at levels more than 5 times as high as those in controls receiving an exchange with albumin alone. Furthermore, constriction of resistant blood vessels and elevation in blood pressure, which are the case with modified hemoglobin, were not observed at all. These are interpreted as implying that, as HbV is of the size to which the vascular wall is impermeable, HbV has little or no effect on nitrogen monoxide (nitric oxide) being an endothelial-derived relaxation factor (EDRF).

The half-life of the HbV in circulating blood was determined to be approximately 35 hours in rats following administration of 25% 99mTc-labeled HbV. It is considered that the administered HbV is captured by Kupffer cells in the liver and macrophages in the spleen, and undergo the same metabolic pathways as those of erythrocytes.

In rats injected with HbV in a dose of 20 ml/kg, exploration of the metabolic process in the reticuloendothelial system and blood biochemical tests disclosed a transient increase in

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**Table 1 Specifications of the Hemoglobin Vesicle**

<table>
<thead>
<tr>
<th>Item</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size (nm)</td>
<td>240–280</td>
</tr>
<tr>
<td>( P_{50} ) (torr)</td>
<td>27–34</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>10.0 ± 0.4 (8.6 ± 0.4*)</td>
</tr>
<tr>
<td>Total lipid (g/dl)</td>
<td>5.3 to 5.9 (4.6 to 5.4*)</td>
</tr>
<tr>
<td>Hb/total lipid (g/g)</td>
<td>1.6–2.1</td>
</tr>
<tr>
<td>PEG-lipid (mol%)</td>
<td>0.3</td>
</tr>
<tr>
<td>metHb (%)</td>
<td>&lt;3</td>
</tr>
<tr>
<td>HbCO (%)</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Viscosity (cP at 230s(^{-1}))</td>
<td>2–3 (3–4*)</td>
</tr>
<tr>
<td>Crystalloid osmotic pressure (mOsm)</td>
<td>300</td>
</tr>
<tr>
<td>Colloidal osmotic pressure (torr)</td>
<td>0 (20*)</td>
</tr>
<tr>
<td>pH (37°C)</td>
<td>7.4</td>
</tr>
<tr>
<td>Endotoxin (EU/ml)</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Sterility test</td>
<td>None detected</td>
</tr>
</tbody>
</table>

*After mixing with a 20% recombinant human serum albumin preparation
PEG: Polyethylene glycol

Therefore, concomitant use of a substance such as human serum albumin (recombinant) is required. As the particle diameter is strictly adjusted to 250 nm, corresponding to about 1/30 that of the red blood cell, a function of which erythrocytes are devoid may be expected, e.g., passage through a site of infarction.

The affinity for oxygen is adjustable to a desired value by co-encapsulating an allosteric effector such as pyridoxal-5'-phosphate (PLP). The composition and contents of lipids are uniquely devised, and the problems inherent in the conventional vesicles have been resolved, including preservability in the liquid state for 2 years at room temperature, avoidance of hemolysis in bloodstream, an appropriate in-blood retention time (i.e., estimated to be about 3 days in humans), and avoidance of platelet and complement activation.

The following are brief accounts of results of the studies conducted to assess the HbV heretofore obtained. The studies were performed mostly in rats and hamsters, and have sufficiently verified the basic safety and oxygen-carrying effect. A safety study in primates is in progress at present.

In rats with 90% of the total blood volume replaced with albumin solution alone to explore the oxygen-carrying effect, decreases in systemic blood pressure and renal cortical oxygen partial pressure became conspicuous after an approximately 70% exchange, resulting in death. When the exchange was carried out using a system consisting of HbV dispersed in albumin solution, both the systemic blood pressure and renal cortical oxygen partial pressure were maintained even after a 90% exchange. In an 80% exchange transfusion study with HbV dispersions in albumin solution conducted in hamsters, tissue oxygen partial pressure in the subcutaneous microcirculation system, as measured non-invasively, was noted to have decreased to 60–70% of pre-exchange value, yet it was maintained at levels more than 5 times as high as those in controls receiving an exchange with albumin alone. Furthermore, constriction of resistant blood vessels and elevation in blood pressure, which are the case with modified hemoglobin, were not observed at all. These are interpreted as implying that, as HbV is of the size to which the vascular wall is impermeable, HbV has little or no effect on nitrogen monoxide (nitric oxide) being an endothelial-derived relaxation factor (EDRF).

The half-life of the HbV in circulating blood was determined to be approximately 35 hours in rats following administration of 25% 99mTc-labeled HbV. It is considered that the administered HbV is captured by Kupffer cells in the liver and macrophages in the spleen, and undergo the same metabolic pathways as those of erythrocytes.

In rats injected with HbV in a dose of 20 ml/kg, exploration of the metabolic process in the reticuloendothelial system and blood biochemical tests disclosed a transient increase in
weights of the liver and spleen, and that HbV taken up by phagocytes disappeared almost completely in a week. There was no evidence of any particular abnormality in hepatic or renal function. Blood lipase level showed a significant elevation transiently, while there was no change in blood amylase level. Serum lipid components, especially cholesterol, rose during the metabolic process, and returned to normal levels 7 days afterwards.

In a rat, 14-day, repeated-dose study (10 ml/kg/day) with an ensuing 14-day recovery phase observation, all animals survived (n = 14) throughout the study and post-treatment observation periods. During these periods, the rats continued to exhibit uninterrupted weight gain with no appreciable change in blood biochemical parameters except for transient increases in lipids and lipase. These latter parameters returned to normal levels in 14 days.

Removal of endogenous carbon monoxide, overproduction of bilirubin, and depressed bile secretory function in the liver occurred with hemoglobin, whereas with HbV, no such effects were observed in the said metabolic organ. Hemoglobin becomes incapable of binding oxygen when the heme iron is oxidized from bivalent to trivalent on autoxidation or reaction with active oxygen species. The metHb liberates Fe$^{3+}$ ion which induces Fenton reactions, thereby catalyzing generation of hydroxy-radicals. In the case of HbV, on the other hand, it has been demonstrated in vitro that reactions with which active oxygen species are associated have no influence external to HbV inasmuch as the hemoglobin is encapsulated with lipid bilayer. Further investigation is needed to elucidate the fate of the iron derived from the HbV metabolized in the reticuloendothelial system.

**Summary and Conclusion**

The present objective of artificial red blood cells consists in a complement to transfusion therapy for emergency lifesaving. Upon fulfilling the purpose, artificial red blood cells are relatively rapidly metabolized in metabolizing organs to be replaced with autologous erythrocytes. Moreover, the albumin-heme complex comprising recombinant albumin and a conjugated heme derivative is an oxygen carrier possessing a colloidal osmotic pressure. Its application to new oxygen therapy by taking advantage of its smaller particle diameter than HbV is also anticipated. In addition, cutting-edge research on artificial platelets is also under way by the study group headed by Prof. Yasuo Ikeda at Keio University School of Medicine, with in vivo studies already in progress.

Thus, research on artificial red blood cells and artificial platelets in Japan are progressing chiefly with the Grants-in-Aid for Health-Labour Science Research. These efforts may not only contribute to future medical care in Japan, but also lead to a considerable international contribution for many countries where safe blood supplies are falling short. For now, the long-term development is expected to be promoted in the private sector with a view to benefiting all humanity, though profitability will have to be secured.

**REFERENCES**


