Bovine haemoglobin-based oxygen carrier for patients undergoing haemodilution before liver resection


Summary
We have studied the use of ultrapurified polymerized bovine haemoglobin (HBOC-201) in patients undergoing preoperative haemodilution before liver resection. After autologous blood donation of 1 litre, 12 patients (six males, six females, mean age 59 (35–69) yr) received Ringer’s lactate solution 2 litre and, in a random design, 6% hydroxyethyl starch 70 000/0.5 (HES) 3 ml kg⁻¹ or HBOC-201 0.4 g kg⁻¹ within 30 min. Blood samples were obtained for blood chemistry, co-oximetry, haematology, coagulation profiles and immunology examinations before operation, on the day of surgery, on days 2–4 and 7 after operation, on the discharge day and 3 months after operation. There were no differences in patient characteristics, blood loss, amount of solutions infused, transfused allogeneic blood or duration of hospital stay. There were no local or systemic allergic reactions with infusion of HES or HBOC-201. Patients receiving HBOC-201 developed more pronounced leucocytosis and reticulocytosis during the early postoperative days compared with HES-treated patients. The mean maximum plasma haemoglobin concentration was 1.0 (sd 0.2) g dl⁻¹ at the end of infusion of HBOC-201. The mean intravascular half-life of HBOC-201 was 8.5 h. Patients in both groups experienced temporary changes in liver enzymes and coagulation variables which returned to normal before discharge. Urinalysis revealed no difference between groups and no free haemoglobin was detected in urine. Patients receiving HBOC-201 showed no IgE and only a slight increase in IgG to HBOC-201 on the day of discharge; these were not detectable at 3 months. Single-dose administration of HBOC-201 was well tolerated by patients undergoing elective liver resection surgery and appears to be safe as a substitute during preoperative haemodilution. (Br. J. Anaesth. 1998; 80: 189–194)

Keywords: blood, substitutes; blood, replacement; blood, haemoglobin; fluids, i.v.; fluid therapy; blood, haemodilution; surgery, hepatic

Technical progress in the purification and formulation of haemoglobin solutions has resulted in the production of oxygen carriers which are free of toxic side effects on the liver and kidneys. Animal studies in different species have shown that a novel ultrapurified polymerized bovine-derived haemoglobin does not cause severe side effects on the liver, kidney or coagulation, even in doses higher than those used clinically. In addition, this bovine haemoglobin solution has a calculated relative potency of three times that of red blood cells (RBC) in restoring tissue oxygenation. A phase I study in normal volunteers showed that polymerized bovine haemoglobin had a dose-dependent intravascular half-life of 16 h.

Several studies have shown that patients with different tumours had a shorter disease-free survival and higher incidence of recurrent or metastatic cancer if they received perioperative allogeneic blood transfusions. There is also evidence that the rate of postoperative infection is higher in patients after homologous red cell transfusion than in patients who were not transfused. As a consequence, perioperative blood substitution with haemoglobin solutions may be advantageous in cancer patients by reducing the amount of allogeneic blood transfusions. As animal data indicate that bovine haemoglobin could enhance the amount of preoperatively donated blood this material may have an indication in cancer patients who are normally excluded from predonation of autologous banked red cells.

This prospective, randomized study was designed to examine the safety and tolerance of a moderate dose of bovine haemoglobin compared with hydroxyethyl starch when used during acute preoperative blood donation in patients undergoing liver resection.

Patients and methods
After obtaining Ethics Committee approval and informed consent, we studied 12 patients (six males, six females, mean age 59 (range 35–69) yr, ASA I–II) undergoing elective liver resection surgery. Exclusion criteria were severe cardiovascular disease (e.g. uncontrolled hypertension >180/100 mm Hg, recent myocardial infarction <6 months, unstable angina, congestive heart failure), decompensated pulmonary disease or porphyria. Patients suffering from acute or chronic hepatic infections (e.g. hepatitis B and C), liver cirrhosis, anaemia (preoperative packed cell volume <30% or haemoglobin <10 g dl⁻¹) or allergic reactions to beef products were also excluded.
All patients underwent a screening examination 1–3 days before operation. On the day of surgery, patients were premedicated with midazolam 7.5 mg orally, 1 h before arriving in the anaesthesia room where they were monitored by ECG, non-invasive arterial pressure measurement and pulse oximetry. General anaesthesia was induced with fentanyl 3 μg kg\(^{-1}\), etomidate 0.3 μg kg\(^{-1}\) and vecuronium 0.1 mg kg\(^{-1}\). After tracheal intubation the lungs were ventilated mechanically with 70% nitrous oxide in oxygen. Anaesthesia was maintained with 0.6–0.8 vol% isoflurane and repeated i.v. injections of fentanyl 0.1 mg and vecuronium 0.1 mg kg\(^{-1}\) after 5 min of centrifugation (4000 rpm, Heraeus Sepatech) of arterial blood which was stabilized with ethylenediamine tetra-acetic acid (EDTA). Total haemoglobin concentration was also measured in EDTA-stabilized arterial blood by the haemoglobin cyanide method. Haematology, white blood cell differential and platelets were measured in a standardized blood smear of arterial EDTA-stabilized blood samples by microscopy. Coagulation variables (Quick’s value, activated partial thromboplastin time (APTT) and fibrinogen) were measured by specific tests. A Hitachi 747 autoanalyser was used for measurement of blood chemistry. At every measurement time (MP 1–8), 10 ml of urine were aspirated from the urinary catheter and examined for free haemoglobin concentration, pH, glucose, protein, ketones, red and white cells, epithelial cells and casts. Immunological samples were obtained on the day before surgery, on postoperative day 7, on the day of discharge and 3 months after surgery.

Blood loss, and amount of crystalloids and colloids infused were monitored during operation and the amount of transfused allogeneic blood within postoperative days 1–7. All patients received their own donated blood when intraoperative haemoglobin concentrations were < 7.5 g dl\(^{-1}\) or at the end of operation. Duration of postoperative artificial ventilation in the ICU and duration of hospital stay were recorded.

All measurements were performed before surgery (MP 1), 4 h after surgery in the ICU (MP 2), on the morning of postoperative days 2–4 and 7 (MP 3–6), and on the day of discharge (MP 7). A follow-up examination was performed 3 months after operation (MP 8) (fig.1).

Data are reported as mean (sd). Patient data in both groups were tested by the Mann–Whitney U test. Differences within groups were tested by ANOVA for repeated measurements and post hoc comparison by Student’s t test. Differences between groups were tested by the Wilcoxon test or two-way ANOVA and post hoc Bonferroni’s correction for alpha. All differences were considered significant at \(P < 0.05\).

### Results

Patient characteristics and data on perioperative fluid management are given in table 2. After autologous bovine haemoglobin (HBOC-201, Biopure, Cambridge, USA) 0.4 g kg\(^{-1}\) (group 2) (table 1).

Blood-gas tensions were measured with an ABL 505 (Radiometer, Denmark). Oximetry was performed using the OSM 3 (Radiometer, Denmark). Plasma haemoglobin concentrations were measured after 5 min of centrifugation (4000 rpm, Heraeus Sepatech) of arterial blood which was stabilized with ethylenediamine tetra-acetic acid (EDTA). Total haemoglobin concentration was also measured in EDTA-stabilized arterial blood by the haemoglobin cyanide method. Haematology, white blood cell differential and platelets were measured in a standardized blood smear of arterial EDTA-stabilized blood samples by microscopy. Coagulation variables (Quick’s value, activated partial thromboplastin time (APTT) and fibrinogen) were measured by specific tests. A Hitachi 747 autoanalyser was used for measurement of blood chemistry. At every measurement time (MP 1–8), 10 ml of urine were aspirated from the urinary catheter and examined for free haemoglobin concentration, pH, glucose, protein, ketones, red and white cells, epithelial cells and casts. Immunological samples were obtained on the day before surgery, on postoperative day 7, on the day of discharge and 3 months after surgery.

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<table>
<thead>
<tr>
<th>Table 1 Specifications of HES and HBOC-201</th>
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<tr>
<td><strong>HES</strong></td>
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<tr>
<td>Molecular weight</td>
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<tr>
<td>Osmolarity (mosmol kg(^{-1}))</td>
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<td>Viscosity (mPa s)</td>
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<td>pH</td>
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<td>Viscosity (mPa s)</td>
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<td>Calcium (mmol litre(^{-1}))</td>
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<td>Endotoxin (EU ml(^{-1}))</td>
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<td>Phospholipid (nmol ml(^{-1}))</td>
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<table>
<thead>
<tr>
<th>Table 2 Patient characteristics and perioperative data (mean (sd) or number)</th>
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<tr>
<td><strong>HES</strong></td>
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<tr>
<td>Age (yr)</td>
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<td>Sex (M/F)</td>
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<td>Height (cm)</td>
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<td>Weight (kg)</td>
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<td>ASA class I/II</td>
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<td>Operation time (min)</td>
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<td>Blood loss (litre)</td>
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<td>Crystalloids (litre)</td>
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<td>Colloids (ml)</td>
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<td>RBC (ml)</td>
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blood donation, patients in group 1 received HES 241 (29) ml and in group 2 HBOC-201 233 (44) ml. No local or systemic allergic reactions were detected during infusion of HES or HBOC-201. Two patients in each group underwent resection of one or two liver segments; two patients in group 1 and three patients in group 2 received a left or right hemi-hepatectomy. Because of unexpected tumour spread, resection was not possible in two patients in group 1 and in one patient in group 2.

There were no differences in heart rate, ventilatory frequency, temperature, pulse oximetry or blood-gas tensions between groups at any time. However, on admission to the ICU (MP 2), patients in group 2 had a higher mean arterial pressure than those in group 1 (95 (18) vs 83 (11) mm Hg; P = 0.037).

Plasma haemoglobin concentration peaked at 1.0 (0.2) g dl\(^{-1}\), 30 min after the start of infusion of HBOC-201 and showed a decrease to 0.22 (0.1) g dl\(^{-1}\) on the morning of day 2 (table 3). The extrapolated intravascular half-life of HBOC-201 was 8.5 h.

In contrast with group 1, the concentrations of total arterial methaemoglobin increased after operation in group 2 with the maximum on day 2. The increase in leucocytes on postoperative days 2–4 and reticulocytosis on day 3 were more pronounced in the HBOC-201 group compared with baseline values than in the HES group (table 3).

Haemodilution and operation-dependent decreases in coagulation occurred; these returned to baseline values within 7 days after surgery (table 4). There were no differences between groups.

There were no differences in blood chemistry between groups (table 4, figs 2–4). All HES patients were negative for both IgG and IgE to HBOC-201. No IgE antibody responses to HBOC-201 were detected in group 2. Five of six patients in group 2 developed clinically insignificant titres to HBOC-201, and IgG was undetectable 3 months after infusion.

Urinary output did not differ between the two groups during the early postoperative period. Free
haemoglobin was not detected in urine over time. Urinalysis did not change in comparison with baseline and there were no differences between groups.

Duration of artificial ventilation was 360 (326) min in group 1 and 245 (317) min in group 2 ($P < 0.55$).

Duration of hospital stay was 12.5 (8) days in group 1 and 14.8 (9.3) days in group 2 ($P < 0.66$). Major adverse events were seen in three patients in group 1 and in two patients in group 2. Adverse events included hyperbilirubinaemia (caused by stenosis of the hepatic duct which required operative revision) and increased serum amylase without clinical pancreatitis in group 1, and atelectasis and hepatic insufficiency (on postoperative day 4 after right hemihepatectomy in a patient with a fatty liver who underwent chemotherapy 4 weeks before operation) in group 2. No adverse event was considered to be associated with donation of HES or HBOC-201. With the exception of the one patient who died of multi-organ failure after hepatic insufficiency 4 weeks after operation, all patients were in excellent condition at the follow-up examination.

**Discussion**

We have shown that a chemically modified ultrapurified bovine haemoglobin administered during preoperative haemodilution was tolerated well in patients undergoing liver resection surgery. However, the study failed to show any advantage of HBOC-201 compared with HES in terms of a sparing effect of allogeneic blood administration in these patients undergoing major abdominal surgery. One explanation for this result is the relatively small dose of HBOC-201, which appears to be too low for perioperative blood substitution in cases of massive blood loss. As the intravascular half-life of haemoglobin appears to be dependent not only on stabilization of the tetramer10 11 but also on the dose of the haemoglobin solution given,12 a single dose of HBOC-201 0.4 g kg$^{-1}$ might have resulted in a shorter intravascular life in this study than has been reported in a dose-escalating study with this material.5 In addition, intraoperative blood loss and perioperative infusions may have diluted the intravascular concentration of bovine haemoglobin. Disappearance into the interstitial and lymphatic space via diffusion has also been reported for intra-molecularly stabilized haemoglobin tetramers. 13 However, the intermolecular stabilization of HBOC-201 with glutaraldehyde resulted in haemoglobin polymers with a molecular size of 64 000–500 000 Da, which is greater than with intra-molecular haemoglobin stabilization and should preclude rapid excretion of this material into the interstitium.

As no free haemoglobin could be detected in urine over time, there is evidence that HBOC-201 was cleared by the reticuloendothelial system (RES). Animal studies have shown that either intra- or intermolecular stabilization of haemoglobin tetramers. However, the intermolecular stabilization of HBOC-201 with glutaraldehyde resulted in haemoglobin polymers with a molecular size of 64 000–500 000 Da, which is greater than with intra-molecular haemoglobin stabilization and should preclude rapid excretion of this material into the interstitium.

As no free haemoglobin could be detected in urine over time, there is evidence that HBOC-201 was cleared by the reticuloendothelial system (RES). Animal studies have shown that either intra- or intermolecular stabilization of haemoglobin tetramers prevents rapid renal elimination. However, a high molecular weight provided by polymerization may enhance uptake into the RES.17 The effect that some of the haemoglobin formulations may have on the RES is unclear at present. Although there are animal data which described immunosuppressive effects when tetrameric human-derived haemoglobin solutions were given in a model of bacteremia or in...
combination with endotoxin, there are no clinical data to suggest that immunosuppression occurs in humans. Our patients showed a transient leucocytosis and reticulocytosis after administration of HBOC-201 but bilirubin concentration was not higher compared with patients who received HES. No clinical correlate for immunosuppression, for example fever, delayed wound healing or concomitant infectious diseases, occurred in our study. However, the number of patients in this study was too small to allow conclusions on side effects of HBOC-201 on the immune system.

The early reticulocytosis on day 3 after operation which was observed in patients after administration of bovine haemoglobin may accord with the uptake of the compound by the RES where it possibly contributes to enhanced production of reticulocytes because of an increased iron supply by HBOC-201.

We have shown that administration of a low dose of HBOC-201 had no toxic side effects on the liver, kidney, pancreas or coagulation, even in patients undergoing liver resection who are very sensitive to toxic influences on these respective organs. Liver enzymes increased to an extent which did not differ from values of patients treated with HES. All results were within the range which normally occurs after liver surgery. As animal data have shown that organ toxicity of some haemoglobin solutions may be a function of impurities we did not expect severe side effects on the liver, kidney or coagulation using an ultrapurified haemoglobin preparation. In contrast with an earlier applied stroma-free haemoglobin solution which resulted in impaired renal function as a result of clinically relevant concentrations of endotoxin and phospholipids, endotoxin and phospholipids in HBOC-201 are undetectable.

One common problem with the use of haemoglobin solutions is oxidation of the free oxyhaemoglobin to methaemoglobin which does not contribute to oxygen transport. This may occur in vitro during storage and also in vivo after infusion. The HBOC-201 solution has a shelf-life of more than 2 yr when stored at room temperature and contains a maximum methaemoglobin concentration of ≤10% if the bags are not damaged. However, after the material was given to patients, the fraction of methaemoglobin increased slightly in the HBOC-201 group but was still within the physiological range. It is therefore unlikely that this moderate increase in total methaemoglobin fraction is clinically relevant. Even after a dose of HBOC-201 45 g, the methaemoglobin concentration did not exceed 1.8% in volunteers. HBOC-201 in the oxy-state possesses a very low oxygen affinity which is regulated by chloride ions and not by 2,3-DPG and has been demonstrated to provide adequate tissue oxygen tensions in a model of nearly complete blood exchange. In addition, plasma oxygen transport by HBOC-201 was able to normalize reduced post-stenotic skeletal muscle tissue oxygenation.

The increased oxygen off-loading capacity is a primary property of HBOC-201 and is associated with compensatory haemodynamic changes in order to maintain normal tissue oxygen tensions. Haemoglobin solutions have also been described as having a vasoconstrictive effect as a result of binding of nitric oxide, manifested by increased systemic vascular resistance and possibly by reduced blood flow to specific organs. However, animal data indicate that autoregulatory mechanisms can override vasoconstriction in vitro, for example on coronary arteries. In a rat model, Gulati, Sharma and Burhop showed that chemically modified (e.g. diaspirin cross-linked) haemoglobin did not alter organ blood flow.

Vasoconstriction with increased mean arterial pressure (MAP) and decreased cardiac output has been demonstrated in patients undergoing preoperative haemodilution with HBOC-201 before vascular surgery. This is consistent with our findings of an increased MAP on admission of patients to the ICU. Although there is no evidence that the vasoconstrictive effect of haemoglobin solutions is dose dependent, no haemodynamic differences were seen in this study on day 2 after operation or later.

It is unclear if haemoglobin solutions also increase gastrointestinal smooth muscle tone. However, there was no evidence of an increased incidence of gastrointestinal adverse events or increased pancreateic sphincter tone in the HBOC-201 group as pancreatic amylase and alkaline phosphatase were not higher than values in HES-treated patients.

All major complications were considered not be associated with either HES or HBOC-201. It is known from the literature that liver resection carries a high rate of postoperative complications.

Although all haemoglobin formulations are composed of modified haemoglobin with some immunogenic potential, administration of HBOC-201 was not associated with allergic reactions. However, the study of Hertzman, Keipert and Chang revealed antigenic properties of polyhaemoglobin while other animal studies suggested low antigenicity of purified and chemically modified haemoglobin.

Acknowledgments
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