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South Africa: clinical experience with Hemopure

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Bovine-derived haemoglobin solution (HBOC-201 – Hemopure®) was first registered for routine clinical use in South Africa for the treatment of adult surgical anaemia. This material was administered to a total of 80 patients as part of a clinical surveillance and educational program from April 2001 until October 2002, and thereafter to a further 256 patients as part of ongoing clinical education and surveillance. This paper reports the clinical details, method of administration, clinical outcome, and safety and efficacy data observed in this cohort of 336 patients. Hemopure was well-tolerated by these patients, with a considerably lower SAE rate observed in this study than was reported in the HAEM 114 and 115 phase III clinical trials. Avoidance of blood was achieved in about 89% of patients, with blood administration limited to patients with large or rapid haemorrhage and those patients who failed to demonstrate recovery of their own red cell mass after Hemopure treatment due to the presence of underlying chronic disease states.

As clinicians became more familiar with the product characteristics and unique physiology of haemoglobin solutions, an increasing tendency has been observed for clinicians to administer Hemopure for its tissue oxygenation effect rather than as a red cell substitute. The role of Hemopure as an enhancer of tissue oxygen delivery and its effect on wound healing, requires further properly controlled randomized prospective studies.

Introduction

HBOC-201 (Hemopure®) is a cell-free polymerized haemoglobin solution that not only carries oxygen in the plasma, but also enhances the ability of native red blood cells to take up and off-load oxygen [1,2]. Studies in an artificial capillary model suggest that the free haemoglobin molecules in the plasma facilitate the diffusion of oxygen into the pulmonary capillary blood more rapidly and more efficiently than normal diffusion across the alveolar–capillary barrier and the red blood cell membrane [3,4]. HBOC-201 is manufactured from a plentiful and well-controlled source material, bovine haemoglobin. Only cattle from the United States are utilized as donors, and an extensive herd-management programme ensures that only certified disease-free animals less than 30 months of age are used to provide the haemoglobin. The extensive extraction and purification process used in production has been validated for the removal of potential contaminants including plasma proteins, red blood cell stroma, endotoxin, bacteria, viruses and the agents that are thought to cause transmissible spongiform encephalopathies such as bovine spongiform encephalopathy (BSE) and variant Creutzfeldt-Jakob disease (vCJD). This process produces a sterile, pyrogen-free balanced salt solution containing glutaraldehyde cross-linked bovine haemoglobin polymers, which range in size from 130 to 500 Kd and have an average molecular weight of 250 kDa [1,2].

The product's oxygen dissociation curve is right-shifted with a P_{50} of 43 mmHg, compared to 27 mmHg for human haemoglobin. In contrast to human haemoglobin whose oxygen affinity relies on adequate levels of 2,3-bisphosphoglycerate, the affinity of bovine haemoglobin for oxygen is regulated by the concentration of chloride ions in the plasma. It has a dose dependent intravascular half-life of 16–24 h [5]. When stored within a temperature range from 2–30 °C, it is stable for at least 3 years, can be infused directly without reconstitution, and does not require typing or cross-matching.

In vitro studies utilizing an artificial capillary model and mathematical simulations demonstrate that HBOC-201 acts not only as an oxygen-carrying agent in the plasma, but also facilitates the uptake and release of oxygen by the patient's own red blood cells [3]. Furthermore, in a canine model, Hemopure was shown to take up oxygen in the lungs and release oxygen for diffusion into body tissues at a higher rate

than red blood cells. On a gram-for-gram basis, the HBOC-201 haemoglobin was approximately three times more effective than stored or fresh red blood cell haemoglobin at restoring baseline tissue oxygenation following severe acute anaemia [6].

Bovine-derived haemoglobin solution (HBOC-201) was first registered as Hemopure for routine clinical use in South Africa for the treatment of acute adult surgical anaemia. Initial product approved in terms of a section 21 registration was administered to a total of 80 patients as part of an intense clinical surveillance and educational program carried out by Biopure South Africa until October 2002 [7]. Subsequently, a further 256 patients have received Hemopure as a fully registered product as part of ongoing clinical surveillance and education from October 2002 to February 2005. The purpose of this report is to document the results of the clinical surveillance program of all Hemopure administered to patients in South Africa.

Clinical setting and patient selection

Adult surgical patients, with anaemia resulting from the underlying disease process or consequent on surgical treatment, were included for observation and study if they received Hemopure for the treatment of their acute surgical anaemia at the discretion of the treating doctor. More recently doctors have administered the product not only for the management of anaemia, but also for the described effect of improving oxygen delivery to body tissues. The decision to transfuse a patient was made primarily on clinical grounds by the clinician managing the case. In general, all anaemic cases that received Hemopure were considered by their treating doctor to have reached a 'transfusion trigger', and under normal circumstances, a red cell transfusion of at least two units would have been commenced.

Product supply

Biopure S A conducted regular Hemopure training programs for interested doctors. Additional training was also directed at appropriate nursing personnel who might be exposed to product use in the clinical environment. Hemopure was then made available by the company for use in patient management, primarily to physicians who had completed the formal training sessions. Product was occasionally supplied to doctors who had not attended the training courses, if appropriate and when red cells were not an option for lifesaving emergency management of acute anaemia in situations of grave clinical need. The decision to administer Hemopure to a specific patient, and the indication for such use, was made solely by the doctor responsible for the management of the patient.

Once the treating doctor had decided to administer Hemopure, a record was kept of the patient's diagnosis, indication for product usage, surgical procedure if relevant, and quantity of product used. The treating doctor was supplied with product on the specific condition that any SAE (serious adverse event), or mortality that may have ensued, would be reported to the distributor of the Product. Such SAEs were recorded and the opinion of the treating clinician in respect of the relationship of the SAE to Hemopure administration was noted. These SAEs were then investigated and a decision made regarding the possible causal relationship of the SAE to the product.

Adverse events, unless severe and directly product related, were not recorded.

Dosage

An initial dose of 30 g of Hemopure was administered over a 1–3-h period. The decision to administer a second 30 g unit shortly after the completion of the first dose was based upon the clinical response of the patient, the severity of the anaemia being treated, and the level of plasma haemoglobin achieved after administration of the first 30-g dose. Therefore, if a patient achieved clinical stability during infusion of the first unit, a second dose of 30 g was administered on the same day only if the patient manifested clinical signs or symptoms of ongoing anaemia. In the event that the patient's haemoglobin was < 7·0 g/dl or if it was expected to fall to < 7·0 g/dl, a second unit was recommended to follow shortly after the first unit. Plasma haemoglobin level was measured 1 h after the completion of each unit, and thereafter daily during the period of treatment, in order to assist in the decision for the requirement and timing of further infusions of Hemopure.

Further units of Hemopure were administered on the basis of the clinical stability of the patient, paying particular attention to pulse rate, urine output and the presence of fatigue, especially if the red cell haemoglobin was < 8 g/dL and the plasma haemoglobin remained < 0·5 g/dL. The requirement for additional dosing was not based solely on the total haemoglobin and haematocrit of the patient, but rather on the clinical evidence and symptoms of anaemia.

After the first day of treatment, further dosing for daily treatment of anaemia was based on the patient's clinical behaviour, total haemoglobin and plasma haemoglobin levels. The mean number (± SD) of units of Hemopure used in these patients was 2·252 (SD 1·728).

Once the patient's red cell haemoglobin level commenced spontaneous recovery, or if the clinician decided that the normal compensatory mechanisms for anaemia were sufficiently operational not to require further support with an oxygen therapeutic, the 'oxygen bridge' treatment was considered completed, and further Hemopure administration was discontinued.

More recently, it has been noted that doctors with more experience in product usage, have been administering the

product not only for the management of anaemia, but also in specific clinical situations where improved oxygen delivery to the tissues was the primary pharmacological effect desired.

A maximum of seven units of Hemopure per patient was recommended. If the patient remained anaemic to the point of demonstrating clinical instability after the seventh unit of Hemopure, administration of red blood cells, when permissible, was recommended.

Results

Hemopure was administered to a total of 336 patients for the treatment of acute surgical anaemia for one of three reasons:

1 when blood was not an option,

2 in cases where blood avoidance was medically desirable,

3 for reasons of patient or physician preference.

Hemopure has been administered to a total of 336 patients. A total of ~750 units of Hemopure has been administered to patients since the registration of the product. These included 106 general surgical cases, 45 peripheral vascular surgical cases, 56 plastic and reconstructive surgical patients, 37 cardio-thoracic, 16 orthopaedic, 14 acute trauma patients, 15 gynaecological patients and 35 cases in other surgical disciplines, as well as to 12 patients with a medical cause of acute anaemia. Table 1 details the diagnosis and surgical discipline for the 336 patients who received Hemopure since its introduction to South Africa. A mean of 2·252 units was transfused for each patient treated, with a range of one to 12 units. After the first infusion, plasma haemoglobin increased to a mean value of 0·8 g/dl and to 1·2 g/dl after the second infusion.

Of the 336 patients, 36 critically ill patients received Hemopure when blood was not an option. All 36 patients had compelling medical need for transfusion in the presence of:

- prohibitive religious objections (Jehovah's Witnesses –29);
- or unavailability of red blood cells (allo-immunized patients –5);
- or patients with auto-immune haemolytic anaemia (–2).

When used as an alternative to conventional red cell transfusion, Hemopure adequately oxygenated and stabilized patients as demonstrated by clinical behaviour and the vital parameters monitored during the infusion. In no case was blood required for reasons of inadequate initial clinical response or evidence of ongoing anaemic symptoms. Total blood exclusion was achieved in 176 patients of the first 200 patients treated with Hemopure (88% blood exclusion).

Hemopure was used as a direct alternative to red cell transfusion with allogeneic blood in 72 cancer patients, which included 26 breast and 26 colonic resections for malignancy. In the cancer subset of patients a red cell exclusion rate of greater than 95% was observed.

Recently, a number of doctors experienced in the use of the product as a red cell alternative, have elected to treat patients suffering from various forms of acute tissue ischaemia with Hemopure. These decisions were influenced by the preclinical work that demonstrated improved oxygen delivery to tissues by the free haemoglobin in the plasma, as well as the clinicians' own observations of improved tissue viability in patients with acute tissue ischaemia treated with Hemopure.

Reported product-related adverse and serious adverse events

Hemopure has generally been well tolerated. Product-related serious adverse events (SAEs) were rare in uncomplicated patients treated with Hemopure. In contrast, there were more SAEs recorded in more complicated cases, and in most cases these SAE's were due to the severity of the underlying disease process, serious and life threatening co-morbidity or excessive surgical blood loss. Twenty-three of the deaths that were recorded (24) were considered by the treating doctor to be due to a direct consequence of the underlying disease process or due to serious co-morbidity. None of the deaths reported was considered by the treating physician to be either probably or definitely product-related.

After treatment with the first unit of Hemopure, mean systolic blood pressure was noted to increase slightly. This was not considered to be clinically significant. In 5% of patients, a blood pressure rise of greater than 30 mmHg was observed during initial Hemopure administration. In most of these patients, a reduction in the rate of product administration rate abolished this increase in blood pressure. Occasionally, a short-acting calcium antagonist, or beta blocker, or intravenous nitrate was required to control the blood pressure at optimum levels. In no instance was this product-related hypertension a clinical problem, provided these susceptible patients were identified and the above measures were instituted.

Mean oxygen saturation as measured by pulse oximetry was essentially unchanged after treatment with the first unit of Hemopure, provided it was infused over a period longer than 1 h per unit.

In six patients, signs and symptoms of fluid overload, cardiac failure or pulmonary oedema were observed and reported. On investigation, it was found that in each case the practitioner involved had failed to appreciate the volume expansion characteristics of Hemopure. In an attempt to avoid using either blood or the unfamiliar new product, these clinicians had administered large volumes of Ringer lactate and/or colloid (heta-starch), resulting in patients that were more than adequately volume replaced, but who still demonstrated clinical evidence of severe acute anaemia and tissue hypoxia. At this late stage Hemopure was then administered rapidly, usually resulting in prompt resolution of the anaemic manifestations, but with the patients then developing evidence of fluid overload with rising central venous pressure (CVP), pulmonary infiltrate and tachycardia. All six of these patients responded promptly to intravenous diuretics and reduction

Table 1 Number of patients receiving Hemopure® analysed according to surgical discipline and surgical treatment $n = 356$

Diagnosis/discipline	Procedure	Number
General surgery	Various general surgical procedures	**106**
Colonic malignancies	Colonic resection	26
Hepatic malignancy	Hepatic resection	3
Retroperitoneal neoplasm	Resection retroperitoneal mass	4
Lymphoma	Splenectomy	2
Hepatic malignancy	Major hepatic resection	3
Other malignancies	Radical resection	8
Crohn's disease	Small bowel resection	4
GIT bleeding	Treatment of acute anaemia	10
Other general surgical uses	Various operations	46
Peripheral vascular surgery		**45**
Femoropopliteal atherosclerosis	Femoropopliteal bypass graft or endarterectomy	15
Aortic aneurysm	Aneurysm repair	11
Aorto-iliac atherosclerosis	Aorto-iliac or aortofemoral graft	9
Carotid and subclavian atherosclerosis	Carotid and subclavian artery reconstruction	2
Other vascular procdures		8
Acute trauma	Major trauma operations	**14**
Other surgical disciplines	Major surgical procedures	**35**
Plastic and reconstructive surgery		**56**
Carcinoma breast	Mastectomy and reconstruction	26
Reconstructive	Major plastic reconstructions with flaps	30
Cardiac and thoracic surgery		**37**
Coronary artery disease	Coronary artery bypass grafting	15
Cardio-thoracic disease other	Major thoracotomy	22
Orthopaedic	Major orthopaedic procedures	16
Medical indications	Medical intervention and infusion of Hemopure	**12**
Gynaecological	Pelvic/gynae operations	**15**
Blood not an option		**36**
Jehovah's Witnesses		29
Allo-immunized patients		5
Autoimmune haemolytic anaemia		2

of fluid load. Four of the six patients required ventilatory support while the diuresis was effected. In all cases the SAE of fluid overload resolved. This highlights the importance of appropriate physician training prior to the use of Hemopure.

Once administered Hemopure has a half life of 16–24 h. Of particular concern was the observation that several (3) patients with severe and acute anaemia were under-treated after receiving an initial unit, resulting in grave consequences. These patients presented with profound anaemia, and initially had Hemopure administered to relieve life-threatening signs and symptoms of anaemia. The anaemia responded appropriately to the administration of the initial dose of the drug, with the patients becoming clinically stable and well-oxygenated. However, 24–36 h later, when the plasma level of haemoglobin fell, the patients once again manifested evidence of severe anaemia. As a consequence of failure of the treating clinician to administer additional doses of Hemopure, these patients then developed all the adverse consequences of their severe anaemia once again. Each of these three patients then succumbed to the effects of acute uncompensated anaemia. These occurrences highlight the requirement for appropriate physician training in order for this material to be optimally utilized and to permit maximum patient benefit.

Minor skin rash of unknown aetiology was seen in a few patients, none of which resulted in discontinuation of the product. No cases of acute drug allergy or anaphylaxis were noted. No cases of clinically significant met-haemoglobinaemia were encountered. Some patients were noted to develop transient skin pigmentation and colouration of the sclera. Where measured, both the conjugated and unconjugated bilirubin were elevated as expected with the breakdown of haemoglobin. No case of overt hepatic dysfunction was encountered. No cases of untoward fever were seen after administration of Hemopure. No cases of pancreatitis were recorded due to the administration of Hemopure. No reported case of acute myocardial infarction was seen after Hemopure administration. No TIA or stroke was encountered after Hemopure administration. A small number of patients developed abdominal cramps and/or dysphagia within 24 h of receiving Hemopure. Of these, most patients responded well to the administration of a small dose of an anticholinergic such as atropine [7] (up to 0·5 mg IV).

Over the total 336 patients, 5 cases of deterioration of renal function were reported. In each of these cases, it appeared on investigation that the nature of the associated pathology and surgical circumstances explained the occurrence of the renal compromise. In no instance could the renal failure be attributed directly to the administration of Hemopure. Four of the patients required dialysis. All patients recovered to their preoperative renal status.

While Hemopure was administered to the first 80 patients solely as a direct alternative to blood transfusion [8–13], in the most recent 256 patients there has been an increasing tendency for Product to be administered for its tissue oxygenation effect. The role of Hemopure as an enhancer of tissue oxygen delivery [14] and consequent improved wound outcome has been observed and commented upon by several participating doctors, particularly in cases of surgical skin flaps, ischaemic bowel, cardiac ischaemia, acute limb ischaemia and traumatic injuries (see Table 2 for details).

Table 2 Summary of cases receiving Hemopure primarily for the desired effect of improving tissue oxygenation

Nature of cases	Number
Acute upper limb ischaemia	3
Traumatic degloving injury of foot	2
Improvement in skin flap perfusion in breast reconstruction	26
Improvement in skin flap perfusion in plastic surgical procedures	30
Re-implantation of severed hand	2
Necrotizing fasciitis	1
Chronic lower limb ischaemia	2
Acute lower limb ischaemia	5
Acute reversible myocardial ischaemia	3
Acute small bowel ischaemia	4
Acute colonic ischaemia	4
Chronic myocardial ischaemia	2

Discussion

Hemopure is effective as an oxygen therapeutic drug as demonstrated by the avoidance of red blood cell transfusions in the majority of cases treated.

Hemopure, used as an alternative to conventional red cell transfusion, adequately oxygenated and stabilized patients as demonstrated by clinical behaviour and the vital parameters monitored during the infusion. In some instances, clinicians with limited experience with the product were reluctant to rely solely on the haemoglobin solution to treat patients with severe and rapid blood loss, and elected to give red cells early in treatment. In no other instance was there a requirement to supplement Hemopure infusions with red blood cell transfusion for reasons of inadequate *initial* clinical response or ongoing anaemic symptoms.

The postoperative red cell haemoglobin level was noted to rise rapidly over a 2–4-week period in most patients after the administration of Hemopure, a phenomenon reported from the clinical trials [8–10]. In a small group of patients with underlying chronic disorders (rheumatoid disease, pancreatic neoplasm, lymphoma, liver metastases and extensive Crohn's disease) a persistent failure to 'build back' their own red cell mass in the early postoperative period required the transfusion of red cells some days after their initial treatment with Hemopure.

Serious adverse events were rare in uncomplicated patients treated for their surgical anaemia with Hemopure. In contrast, in more complicated cases with more advanced and challenging disease profiles and with more severe associated co-morbidity, more SAE's were observed. In most of these instances, the SAEs were due to the severity of the underlying disease process and the pressing urgency of their general medical condition. Few SAEs, apart from fluid overload, were definitely associated with Hemopure administration. Since Hemopure is a colloid, care should be exercised to avoid fluid overload in the postoperative patient due to receive Hemopure.

The occurrence of 24 deaths at first appears to be of concern. However, after analysis of each case it became apparent that in the majority of these instances,

- the patients had experienced exsanguination as a consequence of surgical misadventure or gravely advanced pathology, and Hemopure was administered as a last resort in a dying patient;
- or patients had succumbed to a progression of their underlying disease process and that the death was totally unrelated to the administration of the drug.

In only one patient was the death unexplained. An elderly male with known coronary artery disease and severe ischaemic rest pain in his leg after a failed vascular surgical procedure, suffered a sudden cardiac arrest 12 h after last receiving Hemopure as an alternative to red cell transfusion for the treatment of surgical anaemia. No cause of death could be established.

Of considerable interest was the total absence of death or serious adverse event in all of the cases where the clinician elected to use Hemopure specifically for the desired effect of improved tissue oxygenation, i.e. as an oxygen therapeutic rather than as a substitute for red cells.

In patients undergoing a mastectomy and immediate reconstruction of the breast in whom Hemopure was used as a red cell substitute in the treatment of acute surgical anaemia, attending surgeons reported improved wound tissue perfusion, presumed to be due to better tissue oxygenation. They also reported improved healing of the reconstructed breast skin flaps after administration of Hemopure. This observation of improved wound healing and improved clinical outcome in a group of patients, many of whom require early commencement of chemotherapy to treat their underlying disease process, holds considerable promise and should be the subject of further controlled study.

An important observation has been the frequency with which clinicians have opted to utilize this product primarily as an oxygen therapeutic rather than as a red cell alternative. The prescribing pattern of each of these clinicians has been similar. Almost invariably these have been interested physicians who have completed the formal training course on Hemopure physiology and pharmacology, and who have subsequently had favourable clinical experience with the product prescribed for the registered indication of use as a red cell alternative in the treatment of acute anaemia. Having used the product in this setting and having experienced favourable results with very few adverse events, these clinicians have then opted to utilize the product in circumstances where improved oxygen delivery to the tissues has been the primary clinical indication for treatment. This has included instances of specific local tissue ischaemia in many different clinical settings as set out in Table 2.

It is of considerable interest to note that in the majority of instances where Hemopure has been administered as an oxygen therapeutic for the management of acute tissue ischaemia, the treating doctor has reported beneficial results with improvement in the clinical status of the ischaemic tissues.

These observations strongly suggest that one of the important future clinical roles of haemoglobin solutions will be the management of acute reversible tissue ischaemia. As no alternative pharmaceutical agent is currently effective in favourably altering the dynamics of oxygen delivery to the tissues, appropriately structured prospective randomised clinical trials directed at confirming the efficacy of Hemopure in the management of patients with specific acute tissue ischaemia are now required to determine the clinical role of this pharmaceutical agent for ischaemic indication.

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