Review of Publicly Available Reports of Adverse Events Associated with HBOCs

Toby Silverman, M.D., Laurence Landow, M.D., Hon Sum Ko, M.D., and Kimberly Lindsey, M.D.
Office of Blood Research and Review, CBER, FDA
April 2008

This review addresses the following aspects of the safety of HBOCs currently or formerly in development: 1) pressor effects, 2) oxidative effects as manifested by pancreatic changes including pancreatitis/elevations of amylase and lipase, 3) clinical adverse events, 4) hemodynamic data, and 5) *in vivo* methemoglobin formation. This list of considerations is not all inclusive of adverse events that have been reported publicly or in submissions to FDA.

Experience with Early Forms of HBOCs

The textbook entitled "Blood Substitutes," edited by Robert M. Winslow, summarized the history of blood transfusion and the development of Hemoglobin-Based Oxygen Carriers. Dr. Winslow noted that Von Stark (1898) was probably the first to use an acellular hemoglobin solution to treat anemic patients. Stable preparations of hemoglobin in solution were prepared by Sellards and Minot in 1916 and used in very small quantities in 33 patients with no adverse effects reported.

In 1937, Amberson reported the use of a hemoglobin solution in a small number of patients. He noted the occurrence in some patients of a very dramatic increase in both systolic and diastolic blood pressure not explained by the volume of product administered. This effect occurred almost immediately, reached maximal blood pressure levels by approximately 2 hours, and persisted for 2-4 hours. The increase in blood pressure was accompanied by a reflex bradycardia.

In 1978, Savitsky published the results of infusion of 250 mL of stroma-free hemoglobin containing 1.2% of the stromal lipid present in an unpurified hemolysate of human red blood cells to eight healthy normal male volunteers. Among the eight subjects, one developed abdominal pain and costovertebral angle tenderness. Other notable findings included bradycardia and a "mild" increase in blood pressure lasting approximately 4-5 hours. There was a decrease in urine output and endogenous creatinine clearance that appeared during the infusion of the stroma-free hemoglobin and lasted for 2-4 hours after the infusion. A mild prolongation of aPTT developed immediately after the infusion.

In other experiments summarized by Winslow, "Many patients did well, but others demonstrated hypertension, bradycardia, oliguria, and even anaphylaxis. These adverse effects were not correlated with specific biochemical properties of the solutions themselves."

Subsequent to these experiments, a number of modifications including removal of stromal components and introduction of polymerization and inter- and intra-molecular

crosslinking led to the development of candidate HBOCs that have been evaluated in more recently performed clinical trials.

Potential Pathophysiological Mechanisms of HBOCs

It is currently hypothesized that the binding of nitric oxide (NO) by acellular hemoglobin is responsible for the pressor effect seen with HBOCs. In his 2004 review, Alayash noted that to counter the pressor effect seen with acellular hemoglobins, many of the current manufacturers have introduced conjugation or polymerization of the hemoglobin molecule in an attempt to reduce the extent of extravasation of the acellular hemoglobin into the perivascular tissues where it binds with NO. He noted, however, that even without tissue extravasation, free hemoglobin would have more than a 500-fold higher ability to scavenge NO than the same amount of hemoglobin within red blood cells. While differences in the degree of vasoactivity may be seen at very low doses, all current HBOC products in or previously in development are vasoactive at the doses proposed for resuscitation or for blood "replacement."

Where hemodynamic data are available, all studied HBOC products have been associated with a decrease in heart rate and a decrease in cardiac output/cardiac index. The pathophysiologic mechanisms underlying the simultaneous decrease in heart rate and cardiac output/index have not been elucidated.

It has also been hypothesized that the gastrointestinal effects of HBOC are related to scavenging of NO resulting in spasm of the lower esophageal sphincter. In addition, excursions of pancreatic enzymes such as amylase and lipase have been attributed by some to the scavenging of NO resulting in spasm of the Sphincter of Oddi. Alternative hypotheses are described below.

A second important aspect of hemoglobin biology and biochemistry is the redox potential of acellular hemoglobin. Adverse events reported to be associated with the use of HBOCs include gastrointestinal effects, pancreatitis and pancreatic enzyme excursions, and elevations in liver function tests. In preclinical studies neurotoxicity, proinflammatory effects, and cardiac lesions (pathologically demonstrated) have been noted. The effects of various chemical modifications on the integrity and stability of hemoglobin are not known.

The pancreas is very susceptible to ischemia. There are experimental data to show that the pancreas is an organ that is particularly susceptible to changes in the microcirculation. Ischemic injury to the pancreas may occur during hypotensive episodes with inadequate tissue perfusion, and pancreatic ischemia due to hypoperfusion has been hypothesized to play a major role in the progression from edematous pancreatitis to the inflammatory form. Further, oxygen free radicals have been reported to result in oxidative stress in the pancreas contributing to the development and progression of acute pancreatitis. The role of NO in experimental pancreatitis has been paradoxical. Some authors report a beneficial effect of NO in pancreatitis, while others report a detrimental effect. There are

no clinical data that demonstrate the nature of the relationship between systemic NO levels and the severity of acute pancreatitis.

Pancreatitis has been reported to complicate massive hemolysis. Druml performed a retrospective analysis of 40 cases of massive hemolysis, defined by decrease in hematocrit by >12%/12 hours, and found that 25% of such cases were associated with pancreatitis. Pathologic changes ranged from mild inflammation and edema to patchy parenchymal necrosis, focal bleeding, and massive swelling. Plasma free hemoglobin levels ranged from a low of 38 mg/dL to a high of 3900 mg/dL. There have been a number of case reports documenting episodes of acute pancreatitis complicating massive hemolysis with the conclusion that acute pancreatitis is an often unrecognized complication of hemolytic anemia.

Another possible basis of HBOC toxicity is the rate at which HBOC products are oxidized to methemoglobin *in vivo*. This aspect of HBOC biology has the potential to limit the clinical usefulness of these products, particularly in patients requiring prolonged administration or high doses of HBOCs, as might be expected for blood avoidance or in patients who object to blood transfusion.

More Recent Experience with HBOCs

With these considerations in mind, each of the manufacturers evaluated effects of candidate HBOCs on hemodynamic parameters during the course of early phase 1 studies. In some cases, early candidate products were associated with unacceptable safety profiles necessitating chemical modifications to the products. In subsequent phase 2 and phase 3 studies, issues of safety and toxicity of these candidate HBOCs have emerged.

Of eight products that have been studied more recently in humans, data are available in the public domain for six. The data that could be found in an extensive search of publications and public notices have been compiled in Table II. Appendix I contains definitions of adverse events, consistent with MedDRA terminology, that were used in this analysis. Some caveats must be kept in mind when reviewing the data included in this report:

1. Not all clinical trials conducted by commercial sponsors have been published.
2. The results listed here are not synonymous with line listings of the type that would be reported to FDA in a comprehensive final study report.
3. For each paper, editorial decisions have been made about what information should be included and what information should be excluded. Aggregating information to derive a comprehensive list of adverse events may not be possible and may not give a completely accurate tally of all adverse events that occurred.
4. Although data on treated and control subjects have been aggregated, it is important to note that not all studies were controlled.
5. While every effort has been made not to count a subject more than once for each category of events (row listing), it is possible that subjects may have been counted twice because of the reporting methods used in the publications.

6. Not all enzyme elevations were captured as adverse events.
7. The number of subjects experiencing enzyme elevations in the clinically significant ranges was not uniformly captured and in some cases was not reported. In some instances, only means and standard deviations, not number of subjects contributing to the dataset, were captured.
8. In some instances, the number of subjects was calculated from reported percentages. In these circumstances, the denominator was assumed to be the number of subjects in each cohort. This assumption may not be correct.

The sources of data were as follows:

- For Biopure, only surgery studies are included. Results are as cited in posted materials from the December 2006 meeting of the Blood Products Advisory Committee [www.fda.gov/ohrms/dockets/ac/cber06.html#BloodProducts]
- For Northfield, information from press releases and meeting presentations has been used as sources of information.
- For Apex and Enzon, no published information is available
- For Baxter, Hemosol, Sangart, Northfield, and Somatogen, information has been obtained from the papers cited in the bibliography in Appendix II.

The following abbreviations are used in Table I:

T test group
C control group
* not reported or no information

Table I. Summary of Adverse Events Reported in the Literature or Publicly Available

	Apex		Baxter		Biopure		Enzon		Hemosol		Northfield		Sangart		Somatogen	
Cohort	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**
Number of Subjects	**Not reported**		**433**	**439**	**708**	**618**	**Not reported**		**209**	**192**	**623**	**457**	**85**	**45**	**64**	**26**
1 Death	*	*	65	44	25	14	*	*	1	4	73	39	2	0	*	*
2 Hypertension	*	*	76	38	166	59	*	*	113	75	*	*	7	1	8	0
3 Pulmonary Hypertension	*	*	1	0	3	0	*	*	*	*	*	*	*	*	*	*
4 Chest pain/chest tightness	*	*	*	*	21	16	*	*	*	*	*	*	*	*	6	0
5 Congestive heart failure	*	*	0	1	54	22	*	*	0	2	17	20	*	*	*	*
6 Cardiac arrest	*	*	*	*	17	6	*	*	1	1	14	9	*	*	*	*
7 Myocardial infarction	*	*	6	1	14	4	*	*	14	7	29	4	2	0	*	*
8 Cardiac arrhythmias/ conduction abnormalities	*	*	23	17	153	100	*	*	1	1	*	*	15	5	1	1
9 Cerebrovascular accident, cerebrovascular ischemia, TIA	*	*	*	*	16	3	*	*	2	1	3	1	*	*	*	*
10 Pneumonia	*	*	*	*	35	22	*	*	*	*	27	21	*	*	*	*
11 Respiratory distress/ failure	*	*	*	*	22	12	*	*	*	*	21	17	*	*	*	*
12 Acute renal failure	*	*	1	3	10	4	*	*	2	2	*	*	*	*	*	*
13 Hypoxia, cyanosis, decreased oxygen saturation	*	*	*	*	76	35	*	*	1	1	*	*	*	*	3	1
14 Hypovolemia	*	*	*	*	19	4	*	*	*	*	*	*	*	*	*	*
15 Gastrointestinal	*	*	51	31	345	195	*	*	23	1	*	*	57	20	36	6
16 Liver, LFTs abnormal	*	*	27	8	20	5	*	*	8	0	*	*	*	*	6	3
17 Pancreatitis	*	*	11	0	5	3	*	*	1	0	*	*	*	*	*	*
18 Coagulation defect, thrombocytopenia, thrombosis	*	*	*	*	45	17	*	*	1	0	13	4	*	*	*	*
19 Hemorrhage/bleeding/anemia	*	*	33	22	108	55	*	*	1	1	20	17	*	*	*	*
20 Sepsis, septic shock, MOF	*	*	2	2	15	6	*	*	0	1	26	20	*	*	*	*
21 Pancreatic enzyme inc	*	*	13	4	3	0	*	*	*	*	*	*	*	*	*	*
22 Lipase increase	*	*	29	9	48	12	*	*	19	2	*	*	8	4	7	1
23 Amylase increase	*	*	48	45	*	*	*	*	35	20	*	*	7	2	4	1

Appendix I. Adverse events categories- terms included in each category

1. Death
2. Hypertension, blood pressure increased, hypertensive crisis, systolic hypertension, systemic vascular resistance (SVR) increased, malignant hypertension, postoperative hypertension, systolic blood pressure increased
3. Pulmonary Hypertension
4. Chest pain, chest tightness
5. Congestive failure- cardiac, cardiac failure, cardiorespiratory failure, left ventricular failure, pulmonary edema, acute circulatory failure, rales, cardiac index decreased, cardiac output decreased, central venous pressure (CVP) increased, fluid overload, dyspnea
6. Cardiac arrest, cardiopulmonary arrest, Ventricular fibrillation
7. Myocardial infarction
8. Arrhythmias and conduction abnormalities
9. Cerebrovascular accident, cerebral infarction, hemiparesis, hemiplegia, monoparesis, transient ischemic attack, reversible ischemic neurological deficit (RIND), transient cerebrovascular event,
10. Pneumonia, pneumonia-Klebsiella, pneumonia-pseudomonas, pneumonia-staphylococcus, aspiration pneumonia, pneumonitis
11. ARDS, respiratory distress, respiratory failure.
12. Acute renal failure
13. Hypoxia, decreased oxygen saturation, cyanosis
14. Hypovolemia
15. Gastrointestinal pain, GI pain-upper, esophageal spasm, vomiting, abdominal distension, dyspepsia, dysphagia, eructation, vomiting-aggravated, postoperative nausea, retching, abdominal pain-lower, nausea-aggravated, nausea, ileus
16. LFTs abnormal
17. Pancreatitis
18. Coagulation disorder, DIC, thrombocytopenia, thrombocythemia, aPTT prolonged, Bleeding time prolonged, fibrinogen decreased, D-dimer increased, prothrombin level decreased, petecchiae, purpura, thrombosis, arterial thrombosis-limb, deep venous thrombosis, pulmonary embolus, thromboembolism, thrombophlebitis-deep, PT change.
19. Anemia, duodenal ulcer hemorrhage, gastric ulcer hemorrhage, rectal hemorrhage, exsanguination, ulcer hemorrhage, intraoperative hemorrhage, postoperative hemorrhage, secondary anemia, hemoglobin decreased, vaginal hemorrhage, and anemia aggravated.
20. Sepsis, septic shock, multiple organ failure

Appendix II. Bibliography

General References:

J Cell Comp Physiol 5 (3): 359-382, 1937

Ann Surg 188 (2): 197-201, 1978

Transfusion 48: 574-575, 2008Clin Pharm Ther 23: 73-80, 1978

Ann Hematol 63: 39-41, 1991

Hepatogastroenterology 46(29): 2736-2750, 1999

Drug Metab Rev 31 (4): 971-997, 1999

Ann Surg 231 (3): 352-360, 2000

Nature Rev 3: 152-159, 2004

Transfusion 45: 1691, 2005

Mol Nutr Food Res 49: 1030-1043, 2005

World J Gastroenterol 12 (18): 2914-1918, 2006

Pancreatology 7: 403-408, 2007

Transf Alt. in Transf Med 9: 204-212, 2007

Transfusion 48: 776-782, 2008

Adverse Events and Pancreatic Enzymes:

Apex: No information

Baxter:

American Journal of Kidney Diseases 26(6): 918-923, 1995

Crit Care Med 24(12): 1993-2000, 1996

Crit Care Med 25(9) 1480-1488, 1997

J Hyperten 16: 1459-1465, 1998

Stroke 30: 993-996. 1999.

JAMA 282(19): 1857-1864, 1999

British Journal of Anaesthesia 83(5): 702-707, 1999

Anesthesiology 92(3): 646-656, 2000

Art Cells, Blood Subs, and Immob Biotech 30(4): 259-283, 2002

J Trauma 52: 887-895, 2002

J Trauma 53(5): 1031-1032, 2002

Anesth Analg 97: 323-332, 2003

Intensive Care Med 29: 378-385, 2003

Military Medicine 169(7): 546-550, 2004

Biopure:

Anesthesia Analgesia 83: 921-927, 1996

Anesth Analg 87: 284-291, 1998

Br. J Anaesthesia 80: 189-194, 1998

Br. J Anaesthesia 83(5): 702-707, 1999

J. Vasc. Surg 31(2): 299-308, 2000

Journal of Thoracic and Cardiovascular Surgery 124(1): 35-42, 2002

Anesth. Analg. 94: 799-808, 2002

Expert Opin Biol Ther 393): 509-517, 2003

Blood Products Advisory Committee meeting: December, 2006: FDA briefing document. Transcript available at www.fda.gov

EuroPCROnline.comEuroInterv. 3:600-609, 2008

Enzon: No information

Hemosol:

Crit Care Med 28 (7): 2283-2292, 2000

J. Cardiothoracic and Vascular Anesthesia 16(6): 695-702, 2002

J Am Coll Surg 198: 373-383, 2004

Northfield:

J Trauma 43(2): 325-332, 1997

Am J Surg 176: 612-617, 1998

J A Coll Surg 187: 113-122, 1998

J Am Coll Surg 195: 445-455, 2002

Northfield press releases and presentations available at Northfield website www.northfieldlabs.com

Somatogen:

Art. Cells, Blood Subs., and Immob. Biotech 22(3): 457-465, 1994

Anesthesioloy 86(4): 848-858. 1997

Intraoperative use. Journal of Cardiothoracic and Vascular Anesthesia 15(5): 593-602, 2001

Sangart:

Transfusion Medicine, 18: 28-39, 2008

Anesthesiology: 105(6): 1153-1163, 2006