INTERFACE

SOCIETY FOR TECHNOLOGY IN ANESTHESIA

2743 S. Veterans Pkwy PMB 193 □ Springfield, IL 62704 □ 217-787-3281 □ SocTecAnes@aol.

FALL 1999 □ VOLUME 10 □ NO. 3

ENHANCING OXYGENATION

-alternatives to packed red blood cells-

The 27th annual meeting of the International Society on Oxygen Transport to Tissue included a panel titled, "Enhancing Oxygenation." The panel consisted of scientists from companies developing products designed to enhance tissue oxygenation. The same panelists accepted an invitation to report on the clinical pharmacology of their respective products in this issue of Interface.

The exciting new therapies for improving oxygen delivery to tissues are – Oxygent™ (a perflourocarbon emulsion), RSR13 (a synthetic allosteric Hb modifier), and Hemopure™/ HBOC-201 (a bovine Hb-based oxygen carrier).

In the broadest sense, oxygen transport to tissues is the ultimate goal in the practice of critical care medicine. Virtually all morbid patients outcomes are associated with inadequate oxygen supply, oxygen demand, or oxygen utilization within vital tissues as a result of disease.

Unfortunately, transfusing red blood cells (RBCs) does not reverse many of the pathologic conditions associated with ischemia. Storage defects, viscosity effects, microvascular hypoperfusion, and O2 diffusion limitations are among the reasons RBCs do not improve tissue hypoxia as well as predicted.

In contrast, the new therapies (Oxygent, Hemopure, RSR13) appear to get O2 through narrowed vessels better than tranfused RBCs. Strict indications and contra-indications remain to be defined. However, if these oxygenation therapies indeed prove to be effective, monitors will be needed to provide clinicians better "triggers" for use. Stay tuned.

—George Blike

STA-2000 Program Finalized

Disney's Coronado Springs Resort
Lake Buena Vista, Florida

Thanks to the efforts of the Program Committee, Chaired by Richard Bartkowski, the 10th Annual Meeting of the STA will feature a diverse faculty exploring *Technology for the Next Century.*

See you there!

INSIDE THIS ISSUE:

Hemopure 2
Oxygent 5
RSR13 7
Meetings 11

BIOPURE'S ROOM TEMPERATURE STABLE HEMOGLOBIN-BASED OXYGEN CARRIER, HEMOPURE® (HBOC-201)

Edward E. Jacobs, MD, Maria S. Gawryl, Ph.D., and L. Bruce Pearce, Ph.D.
Biopure Corporation, 11 Hurley Street, Cambridge Massachusetts 02141

INTERFACE is the official newsletter of the Society for Technology in Anesthesia. The newsletter is published quarterly and mailed directly to the membership of the society. The editors invite suggestions, contributions and commentary about published items. Please send all correspondence to:

George T. Blike, MD
Co-Editor, STA *Interface*
Department of Anesthesiology
Dartmouth-Hitchcock Medical Center
One Medical Center Drive
Lebanon, NH 03756
Phone: (603)650-5597
Fax: (603)650-8980
E-mail: George.Blike@Hitchcock.org

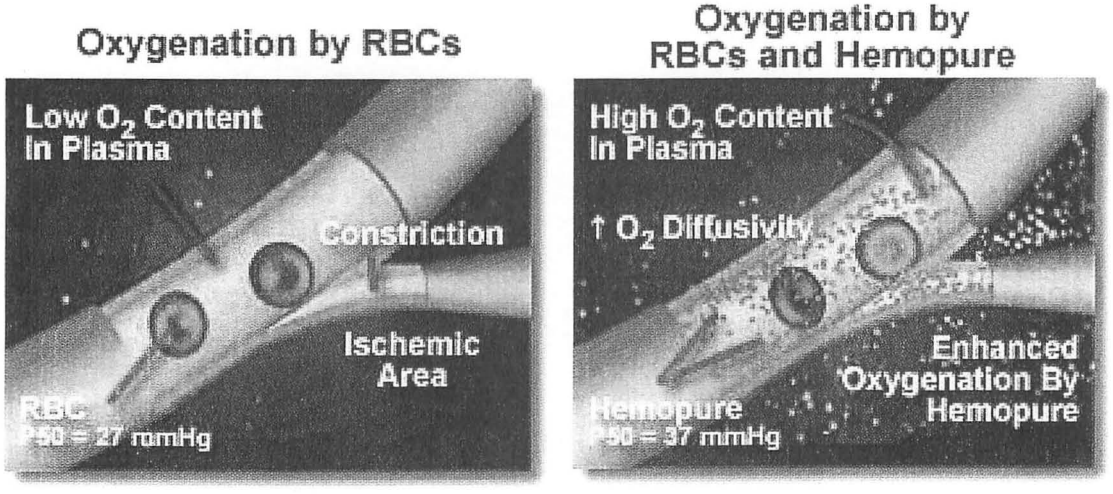
Christopher Wiley, MD
Co-Editor, STA *Interface*
Department of Anesthesiology
Dartmouth-Hitchcock Medical Center
One Medical Center Drive
Lebanon, NH 03756
Phone: (603)650-5864
Fax: (603)650-8980
E-mail: Chris.Wiley@Hitchcock.org

Introduction

Hemoglobin-based oxygen carriers (HBOC), together with perfluorochemicals, comprise a class of pharmacologic agents known as oxygen therapeutics. The various HBOC formulations involve animal or human derived native Hb that has been modified to make these compounds more practical, safe and effective. The approach taken by Biopure Corporation is to ultrapurify bovine Hb from a controlled source and to both intra and intermolecularly cross-link the Hb molecules to produce Hb polymers in physiologic buffer with a highly consistent and defined character. Biopure produces two room temperature stable formulations; (1) Oxyglobin (HBOC-301), the HBOC approved for veterinary use by the FDA in 1998 and the European Union in 1999 and (2) Hemopure, the HBOC intend for human use. Hemopure is being evaluated in a multinational phase III clinical trial.

The pharmacology of these formulations is defined by a complex combination of effects on the rheology of blood and the efficiency of oxygen transport. The primary pharmacodynamic effects of Hemopure, however, are associated with oxygen transport from the lung to the tissues. Hemopure improves oxygenation by enhancing both convective and diffusive oxygen transport as depicted in Figure 1 (animated at web site: http://www.biopure.com/education/animations_home.htm). Convective transport refers to the movement of oxygen-laden Hb in blood, and in the case of Hemopure, can be achieved in three ways. First, Hemopure acts as a plasma volume expander, promoting tissue and organ perfusion. Second, the microvascular rheology of Hemopure contributes to increased flow to and thus oxygenation of tissues by reducing viscosity during hemodilution (viscosity of Hemopure = 1.3 vs 3.8 centipoise for blood at 37°C). Hemodilution to hematocrits as low as 15% has been suggested to be optimal in the cerebral microcirculation (Hudetz et al., 1999). Third, the distribution of Hemopure present in plasma is not limited by the normal microcircula-

(Continued on page 3)



Hemopure

(Continued from page 2)

tory mechanisms that prioritize RBC flux in capillary networks. While mammals have evolved elegant mechanisms for conserving and controlling oxygen delivery to the body's tissues, the perfusion of capillaries by plasma containing HBOC is relatively insensitive to these mechanisms. Accordingly, infusion of even a small amount of Hemopure can produce an increase in the amount of oxygen carrying Hb that enters all capillary beds regardless of the RBC distribution. The latter may be important in disease and tissue injury where mal-distribution of RBCs may be associated with focal or regional hypoxia.

The diffusive component of oxygen transport, specifically the flux of oxygen into and out of blood, is significantly altered by Hemopure. There are two mechanisms by which Hemopure modifies diffusive oxygen transport. The lower affinity of Hemopure (P_{50} = 38 mmHg vs 27 mmHg for RBC Hb) increases the tendency to off-load oxygen to tissues compared to native RBC Hb. The most significant effect on the diffusive component of oxygen transport results from the presence of Hemopure in the plasma. Oxygen is sparingly soluble in plasma and thus the plasma acts as a barrier limiting the transfer of oxygen from RBC Hb to the tissues. The distribution of Hemopure in the plasma phase appears to decrease the magnitude of this resistance by facilitating the transfer of oxygen from RBC Hb to the tissues. Accordingly, both modeling of oxygen fluxes and the observation of oxygen fluxes in *in vitro* studies suggest that Biopure's HBOC solutions transport oxygen more efficiently than red blood cells, and when added to RBCs can also increase the efficiency of red blood cell oxygen transport. Data from direct microelectrode measurement of tissue oxygen tensions in dogs suggest that Hemopure is 3 times more potent than RBCs in restoring tissue oxygenation under severe hypoxic conditions.

More than 150 preclinical studies that were crucial to the development of new formulations have been performed on Biopure's formulations of bovine Hb. These studies defined the toxicity and pharmacology of these formulations as required by regulatory agencies and explored their potential medical applications. The latter remains an exciting and ongoing area of laboratory investigation.

Since 1992, Biopure's HBOC formulations have been tested in 20 clinical trials of which 19 are complete and in which more than 600 subjects have received Hemopure in various dosing regimens. The earliest Phase I clinical trials performed with Hemopure investigated the effects of this HBOC in normal volunteers. In these studies, the pharmacokinetics of Hemopure and the effects of this oxygen carrier on exercise performance were examined. Hughes et al.[9] studied the effect of autologous blood transfusion compared to transfusion with Hemopure on exercise performance in a bicycle exercise stress test. Prior to hemodilution, performance was evaluated at the anaerobic threshold and pulmonary diffusing capacity was estimated using the single breath carbon monoxide technique. In this double crossover trial normal volunteers were then hemodiluted by removing 15% of their estimated blood volume and restoring normal volume with lactated Ringer's solution. Hemodiltuion was followed by either autologous blood transfusion or infusion of Hemopure, and retesting of exercise performance. The results of these studies showed that infusion of 45g Hb in the form of Hemopure resulted in the equivalent exercise performance and pulmonary diffusing capacity as observed with 150g Hb added as RBCs. This apparent 1:3 ratio is similar to the difference in potency reported by Standl et al.[7] in dogs where tissue oxygenation was measured directly.

Although the relative potency of the Hemopure and RBCs was not specifically compared in more recent clinical studies, the results of these studies

(Continued on page 4)

Table 1. Elimination of Allogeneic Transfusion in Surgery

Efficacy	Cardiac Surgery	Aortic Reconstruction	Non-cardiac Surgery
Patients requiring no RBC units at follow-up	34%	27%	43%
Safety			
Mortality Rate (HBOC-201 vs control)	2%* vs 0%	6% vs 8%	7% vs 7%
Serious Adverse Events (HBOC-201 vs control)	30% vs 31%	38% vs 38%	29% vs 26%

Hemopure

(Continued from page 3)

presented in Table 1. show that in postoperative and perioperative settings, including cardiopulmonary bypass, abdominal aortic reconstruction, and noncardiac surgery, that Hemopure can completely eliminate the need for allogenic blood transfusion in 34, 27, and 43% of subjects, respectively.' These three studies in combination with the other clinical trials demonstrated that this oxygen therapeutic is well tolerated under a variety of circumstances. Biopure expects to complete its Phase III pivotal trial studying the perioperative application of Hemopure and initiate Phase I and II clinical trials in new indications during 2000. These additional trials will explore the ability of Hemopure to enhance tumor oxygenation and radiation therapy in cancer patients and will investigate the role of Hemopure's in the treatment of trauma patients.

1. Hudetz AG, Wood JD, Biswal BB, Krolo I, Kampine, JP. Effect of hemodilution on RBC velocity, supply rate, and hematocrit in the cerebral capillary network. *J. Appl Physiol* 1999; 87 (2):505-509.

2. Hellums, JD The resistance to oxygen transport in the capillaries relative to that in the surrounding tissue. *Microvas Res* 1977;13: 131-136.

3. Page, TC, McKay, CB, Light, WR, Hellums, JD. Experimental simulation of oxygen transport in microvessels. *In* RM Winslow, KD Vandegriff, and M Intaglietta, editors. Blood Substitutes: New Challenges. Boston: Birkhauser, 1996, 132-145.

4. Page, TC, Light, WR, McKay, CB, and Hellums, JD. Oxygen transport by erythrocyte/hemoglobin solution mixtures in an *in vitro* capillary as a model of hemoglobin-based oxygen carrier performance. *Microvas Res* 1998; 55:54-64.

5. Page, TC, Light, WR, and Hellums, JD. Prediction of microcirulatory oxygen transport by erythrocytes/ hemoglobin solution mixtures. *Microvas Res* 1998; 56:113-126.

6. Page TC, Light, WR, and Hellums, JD. Oxygen transport in 10 mm artificial capillaries In Oxygen Transport to Tissue XXI . New York: Kluwer Academic/Plenum Publishers, 1999, 715-722.

7. Standl, T, Horn, P, Wilhelm, S, Greim, C, Freitag, M, Freitag, U. Bovine haemoglobin is more potent than autologous red blood cells in restoring muscular tissue oxygenation after profound isovolaemic haemodilution in dogs. *Can. J. Anesth.* 1996; 43: 714-723.

8. Hughes, G.S, Antal, EJ, Locker, PK, Francom, SF, Adams, WJ, Jacobs, EE . Physiology and pharmacokinetics of a novel hemoglobin-based oxygen carrier in humans. *Crit Care Med.* 1996; 24:756-764.

9. Hughes, GS, Yancey, E.P, Albrecht, R, Locker, PK, Francom, SF, Orringer, EP, Antal, EJ, Jacobs, EE. Hemoglobin-based oxygen carrier preserves submaximal exercise capacity in humans. *Clin Pharmacol Ther* 1995; 58:434-443.

10. Levy, J.H., Goodnough, L.T., Greilich, P., et al. A room-temperature stable hemoglobin (HBOC-201) eliminates allogeneic red blood cell (RBC) transfusion in postoperative cardiac surgery patients. American Heart Association 71st Scientific Sessions, Dallas, TX. November 8-11, 1998.

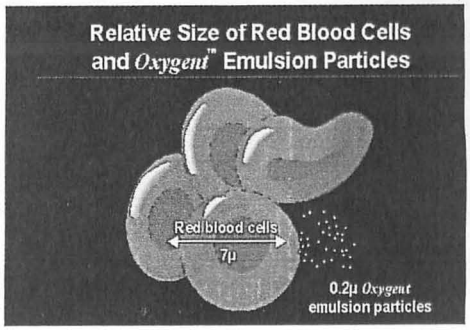
11. Sprung, J., Hoffman, W.D., Gottlieb, A., O'Hara, P.J., Parrett, C. Hemoglobin-based oxygen carrier reduces intraoperative allogeneic blood requirement during abdominal aneurysm repair. Anesth. Analg. 86:S151, 1998

Ξ

Progress in the Development of *Oxygent*: An Intravenous Oxygen Carrier for Use in Elective Surgery

Peter E. Keipert, Ph.D.

Alliance Pharmaceutical Corp., 3040 Science Park Road, San Diego, CA 92121

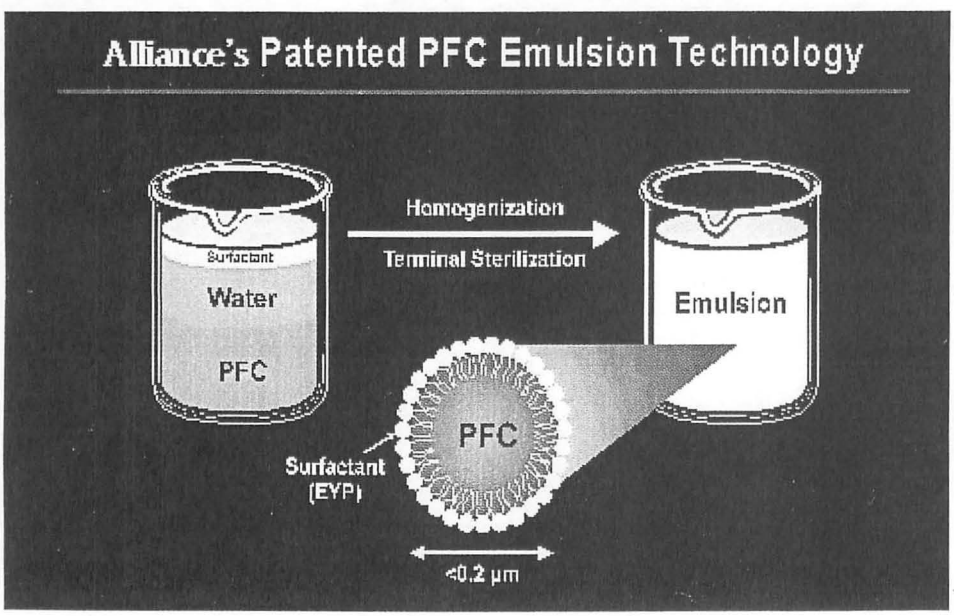


Background:

Perfluorocarbon (PFC) emulsions were first developed over 30 years ago, due to their unique physical and chemical properties which make them ideal temporary intravenous oxygen carriers for clinical indications where tissues may be at risk of inadequate oxygenation. Alliance Pharmaceutical Corp. is presently developing *Oxygent*™; a concentrated 60% w/v emulsion based on perflubron (perfluorooctyl bromide; $C_8F_{17}Br$), which has an initial median particle diameter of 0.16-0.18 μm and is expected to have a shelf-life of up to 2 years when stored at 2°C to 8°C. Each 110-mL unit of *Oxygent* is supplied as a sterilized, ready-for-use product intended for i. v. administration in the acute hospital setting.

Preclinical efficacy:

Several nonclinical efficacy studies have been conducted and have demonstrated that: (1) *Oxygent* can support tissue oxygenation, (2) the oxygen delivered by *Oxygent* is available to support metabolic processes at the tissue level, and (3) this improved oxygenation status can translate into improved organ function.[1] An open chest canine model of cardiac ischemia using *Oxygent*-supplemented cardioplegia solution has shown a direct benefit on overall myocardial oxygenation, tissue metabolic status, and functional recovery after cardioplegic arrest. Studies addressing cerebral tissue oxygenation and cerebral function demonstrated that *Oxygent* may be useful as an anti-hypoxic agent during cardiopulmonary bypass (CPB), which has the potential to cause temporary reductions in cerebral blood flow. Studies in canine CPB models have shown that systemic and myocardial oxygenation, myocardial function recovery, and survival following CPB can be enhanced by adding *Oxygent* to the bypass circuit.



Most relevant to Alliance's current clinical indication are several studies using canine hemodilution models designed to mimic acute surgical anemia and blood loss. To correlate the characteristic PFC-induced increases in blood oxygenation levels (PO_2) with changes in tissue oxygenation, these studies employed polarographic PO_2 electrodes to monitor tissue PO_2 in heart, brain, muscle, gut, and liver. Results demonstrated that improvements in tissue PO_2 in the *Oxygent*-treated animals correlated with the observed increases in oxygenation of mixed venous blood. Moreover, a 1.8 g/kg dose of *Oxygent* was as effective as autologous blood transfusion in protecting tissue oxygenation during volume-compensated blood loss to profound levels of anemia, and was able to maintain better myocardial function (as evidenced by improved left ventricular contractility).[2] Collectively, these nonclinical efficacy studies have confirmed that *Oxygent* can prevent tissue hypoxia and can preserve myocardial and cerebral function.

(Continued on page 6)

Oxygent

(Continued from page 5)

Clinical Indication:

Alliance's objective to first pursue a transfusion-reduction indication is based on the fact that patients and physicians are seeking alternatives to allogeneic blood transfusion. While disease transmission risks are well known and represent the primary reason that patients fear blood transfusions, the availability of donor blood has emerged recently as an equal concern. This appears to be due to a decreasing donor pool (aging population), and an increasing demand for blood for major surgical procedures in older patients. Another concern is related to the apparent immunosuppressive effects of blood transfusion. Hence, there is a renewed interest and urgency in developing alternatives to allogeneic transfusion, especially for surgical procedures, which account for ~ 65% of blood usage. A significant reduction in the demand for blood in this area would help to alleviate the stress on the blood supply, as well as to reduce the risk of transfusion for surgical patients.

Alliance's objective to first pursue a transfusion-reduction indication is based on the fact that patients and physicians are seeking alternatives to allogeneic blood transfusion.

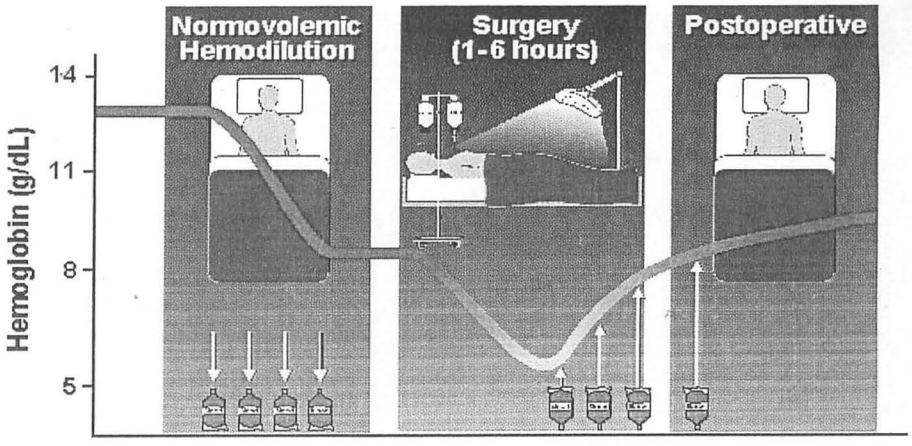


Figure 1.
Augmented-ANH method using *Oxygent* as a temporary oxygen carrier.

Autologous blood strategies, such as preoperative donation, intraoperative salvage, and acute normovolemic hemodilution (ANH; often called intraoperative autologous donation [IAD] in cardiac surgery) all have certain limitations (i.e., either complex logistics, cost, and/or safety constraints). A better approach may be to combine the benefits of ANH or IAD with the use of a temporary oxygen carrier, a technique currently termed Augmented-ANH™ (A-ANH™). This allows the physician to collect more fresh autologous blood at the time of surgery (more red cells available for later reinfusion), and to enhance oxygen delivery with an oxygen carrier during surgical blood loss to temporarily compensate for the additional ANH-induced anemia (see **Fig. 1**). Several potential advantages of employing A-ANH with *Oxygent* include: 1) reducing or eliminating allogeneic transfusions (since fewer red cells are lost during surgical bleeding), 2) providing fresh whole blood with functional platelets and clotting factors, 3) improving safety by decreasing the risk of clerical error, bacterial contamination and immune suppression, and 4) reducing the demand on the limited supply of allogeneic blood.

Clinical Studies:

Completed Phase 1 and Phase 2 clinical studies with *Oxygent* have enrolled a total of 540 subjects. Of these, 340 were randomized to receive *Oxygent*, 65 received a unit of autologous blood, and 135 were volume-matched crystalloid or colloid controls. The safety profile for *Oxygent* has been carefully assessed in several clinical studies involving healthy volunteers (4 studies), cancer patients (6 studies) and surgical patients (2 studies). Collectively, these studies showed that *Oxygent* was well tolerated with no serious adverse events related to drug treatment.

Five Phase 2 studies with *Oxygent* have been completed: these include two large Phase 2b studies in general surgery (n = 246 orthopedic, urologic, and gynecologic patients), and three smaller Phase 2a studies in cardiac surgery (n = 81 patients undergoing coronary artery bypass grafting [CABG] procedures with CPB). In all five studies, in which 160 patients were PFC-treated, *Oxygent* was well tolerated with a good safety profile. These studies

(Continued on page 10)

ALLOSTERIC MODIFICATION OF HEMOGLOBIN BY RSR13 AS A STRATEGY TO ENHANCE TISSUE OXYGENATION

Robert P. Steffen, Ph.D.
Director, Pharmacology and Toxicology, Allos Therapeutics, Inc.
Denver, CO

RSR13, 2-[4-[[(3,5-Dimethylanilino) carbonyl]methyl]phenoxy]-2-methylpropionic acid sodium salt (MW 363) (Figure 1), is a synthetic allosteric modifier of hemoglobin (Hb). RSR13 noncovalently binds in the central water cavity of the hemoglobin tetramer reducing hemoglobin-oxygen affinity [1], described by an increase in p50 (pO_2 for 50% Hb saturation), and enhances the diffusion of oxygen from the blood to the tissues [2, 3, 4]. RSR13 emulates the function of natural allosteric modifiers such as hydrogen ions, carbon dioxide, and 2,3-diphosphoglycerate. By enhancing the release of oxygen from hemoglobin, RSR13 is the first of a new class of pharmaceutical agents to improve clinical conditions characterized by tissue hypoxia due to: 1) inadequate blood flow (regional or global), 2) insufficient oxygen carrying capacity (e. g., hemorrhage or dilutional anemia), 3) increased tissue oxygen demand unmatched by supply (e.g., myocardial ischemia), and/or 4) insufficient oxygen loading/unloading capacity of hemoglobin (e.g., hypothermia).

RSR13 has completed and is currently undergoing a number of animal studies and human clinical trials designed to evaluate the potential clinical utility of this agent in several clinical indications characterized by tissue hypoxia, including radioenhancement in oncology, ischemic coronary artery disease, and surgery/critical care.

Radioenhancement

Radiation therapy is the principle non-surgical means to achieve local control of cancer, with tumor oxygenation playing an important role in the efficacy of radiation therapy. Hypoxic tumor cells are an important cause of radiation treatment failure because of their relative resistance to cell damage by radiation [5]. Tumor hypoxia adversely affects the clinical prognosis of radiation therapy. Oxygen measurements in human tumors have confirmed tumor hypoxia in squamous cell carcinomas of the uterine cervix and head and neck, glioblastoma multiforme, breast carcinoma, and brain metastases. Because hypoxic cells are substantially more resistant to radiation than oxygenated cells, even small hypoxic fractions in a tumor may affect overall response of the tumor to radiation by increasing the probability that some tumor cells will survive radiation treatment.

Animal pharmacology studies have shown that RSR13 dose-dependently increases blood p50 [3, 6, 7], increases pO_2 in non-tumor tissue [3,8,9], and increases oxygen diffusive transport [4] in non-tumor tissue. In animals bearing mammary tumors, tumor pO_2 was measured using Eppendorf histograms with tumor hypoxic fraction expressed as the percentage of readings less than or equal to 5 mmHg. In this model, RSR13 (150 mg/kg) decreased the tumor hypoxic fraction from a control of 36% to 0%, 30 minutes after RSR13 dosing and increased tumor oxygenation [2]. In a mouse model with subcutaneous lung tumors, RSR13 dose–dependently enhanced the efficacy of fractionated radiation therapy, measured as an enhancement of tumor growth delay, by 22%, 40%, and 69% at 50 mg/kg, 100 mg/kg, and 200 mg/kg, respectively. In additional studies, in animals bearing FSaII fibrosarcomas or squamous cell carcinomas[21], or mammary tumors [22], RSR13 decreased tumor cell survival following fractionated radiation. The radioenhancement effect of RSR13 was shown to be oxygen dependent [22], with no direct cytotoxic effect on the tumor, bone marrow [2,22], or skin [22].

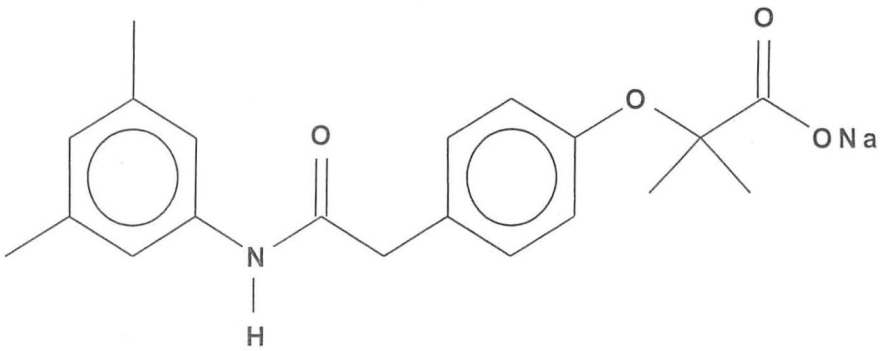


Figure 1. Chemical structure of RSR13 Sodium.

(Continued on page 8)

RSR13

(Continued from page 7)

The radioenhancement effect of RSR13 is not dependent on its entry into the tumor. The effect of RSR13 on hemoglobin in the red blood cell to enhance oxygen release from hemoglobin and the diffusion of that oxygen from plasma and the vascular compartment to the hypoxic tumor cells is the basis for the radioenhancement effect of RSR13. The fact that RSR13 does not have to enter the cancer cell to increase the radiosensitivity of the cancer cells, is an important differentiation between RSR13 and earlier pharmacologic attempts to improve the efficacy of radiotherapy. This is especially important in the setting of primary or metastatic brain tumors, where the blood brain barrier acts to exclude or impede the entry of chemical agents into the brain parenchyma.

Clinical trials were initiated with RSR13 based on its mechanism of action, its ability to enhance the efficacy of radiotherapy in animal models, and its preclinical safety profile. RSR13 has successfully completed a Phase Ia clinical trial in healthy volunteers [10], Phase Ib dose-escalation safety studies in patients with brain metastases requiring palliative radiation therapy [11] or primary brain cancer (glioblastoma multiforme, GBM) [12] undergoing radiation therapy for their cancers. Results have demonstrated encouraging response data and improved survival trends in both patient populations.

In collaboration with the NCI's New Approaches to Brain Tumor Therapy (NABTT) CNS Consortium, a 50-patient Phase II clinical trial with RSR13 in conjunction with radiotherapy in patients with GBM was completed. Preliminary analysis of the Kaplan-Meier survival-curve analysis of the Phase I GBM-patients combined with interim Phase II GBM patients treated with RSR13 in conjunction with standard radiation therapy shows that RSR13 treatment significantly increases median survival compared to the NABTT historical control database.

> By enhancing the release of oxygen from hemoglobin, RSR13 is the first of a new class of pharmaceutical agents to improve clinical conditions characterized by tissue hypoxia

Ischemic Coronary Artery Disease and Surgical Hypoxia

Myocardial hypoxia occurs as the result of an imbalance of oxygen supply to demand, predominantly due to ischemia. Conventional treatment for ischemia-mediated myocardial hypoxia is to increase coronary blood flow or decrease myocardial oxygen demand. By increasing the release of oxygen from hemoglobin, RSR13 may provide and alternate means of reducing myocardial hypoxia. Previous studies assessed the effect of RSR13 on cardiac function and metabolism in animal models of low coronary perfusion pressure and low blood flow myocardial ischemia. Using a rat isolated-heart model under normothermic conditions, Woods [13] reported that RSR13 attenuated the ischemia-induced decrease in high-energy phosphates, adenosine triphosphate, and myocardial creatine phosphate. In an open-chest canine model of myocardial ischemia, Weiss [14] showed that myocardial high-energy phosphates, pH, and fractional shortening were preserved with RSR13, given prior to or following ischemia. Pagel [7] published data demonstrating that RSR13 dose-dependently enhanced the recovery of ischemic bed left ventricular segment shortening throughout reperfusion compared to vehicle-treated animals in a model of coronary artery occlusion- and reperfusion-induced myocardial stunning without affecting function of the non-ischemic circumflex perfused region. In the RSR13-treated animals, the improvement in myocardial contractile function was significantly and positively correlated to the increase in p50.

Significant myocardial and cerebral ischemia may occur in the setting of cardiopulmonary bypass (CPB) surgery. Avoiding ischemic injury during cardiac surgery is dependent on supplying sufficient energy to meet metabolic needs. Although standard clinical cardioplegia and hypothermia provide myocardial protection during surgically-induced ischemia for cardiac surgery, perioperative infarction, stunning, and poor postoperative ventricular function remain significant problems in CPB surgery, especially in high risk patients. Cold cardioplegia has been shown to reduce myocardial energy demand by 95%. However, energy-dependent maintenance of basal cellular metabolism, ionic equilibrium, and membrane integrity is required. Hypothermia not only impairs glycolysis and energy utilization, it reduces oxygen delivery by increasing oxygen binding affinity of hemoglobin. In human whole blood, RSR13 reverses hypothermia-mediated in-

(Continued on page 9)

RSR13

(Continued from page 8)

crease in hemoglobin oxygen binding affinity [15]. In a dog model of hypothermic blood cardioplegia following normothermic cardiac ischemia, RSR13-supplemented blood cardioplegia significantly improved myocardial mechanical function compared to blood cardioplegia alone measured by % return to normal sinus rhythm (100% vs 0%), % of baseline +dP/dt (76.3±1.9 vs 33.7±1.7) and -dP/dt (81.1±1.6 vs 26.6±2.0), stroke volume (7.1±0.9 vs3.5±0.5mL), cardiac output (880±40.3 vs 340±20 mL/min), and LVEDP (0.3±2.9 vs 11.3±2.2mmHg). Indices of myocardial oxidative metabolic function, including myocardial lactate, pyruvate, and ATP content, were similarly improved in the RSR13-supplemented blood cardioplegia group compared to the non-supplemented blood cardioplegia group and myocardial and endothelium morphology appeared better preserved in the RSR13-supplemented cardioplegia group compared to blood cardioplegia alone [16].

Based on the improvement in myocardial function, oxidative metabolism, and evidence of myocyte and endothelial preservation in models of myocardial ischemia, a 30-patient clinical trial with RSR13 in low-risk patients undergoing CPB for first time coronary artery bypass graft surgery was successfully completed. This randomized double-blind, placebo-controlled study demonstrated that RSR13 could be safely administered and provided evidence that patients receiving RSR13 had improved post-surgical outcomes. Cardiac function tended to be improved in the RSR13-treated patients, supported by improved stroke volume and cardiac output. There was a general trend in the RSR13-treated group for less perioperative packed red blood cell use in the RSR13 treated group compared to the placebo treated patients and reduced hospital stay in the RSR13 treated group.

Cerebral Ischemia and Stroke

During focal cerebral ischemia, the magnitude of the infarct size is thought to depend, at least in part, on the physiologic changes within the cerebral penumbra where blood flow is marginal. During the cerebral ischemia and early reperfusion, episodes of depolarization and tissue hypoxia occur, contribution to infarct expansion. By increasing oxygen availability by increasing release of oxygen from hemoglobin, RSR13 may attenuate neuronal damage secondary to cerebral ischemia.

The effect of RSR13 to limit cerebral infarct size under normothermic conditions has been reported. Ischemia-induced cerebral hypoxia was achieved in a cat model by permanent middle cerebral artery occlusion [17]. RSR13 treatment was associated with a significantly smaller cerebral infarct size compared to that in vehicle-treated animals, with a significant inverse relationship between the increase in p50 by RSR13 and the reduction in cerebral infarct size. Consistent with the effect in focal ischemia, RSR13 reduced hippocampal CA1 neuronal cell death by 28% following incomplete global cerebral ischemia in rats [18].

During and after cerebral ischemia, in addition to being hypoxic, the penumbral area is characterized by an excess of glutamate release, a neurotoxic neurotransmitter. Inhibition of glutamate neurotoxicity by the N-methyl-D-aspartate receptor antagonist dizocilpine has been shown to reduce brain damage following focal cerebral ischemia. RSR13 was evaluated in the setting of focal cerebral artery occlusion and reperfusion in combination with dizocilpine. The combination treatment was shown to decrease cerebral infarct size better than dizocilpine alone, when given prior to ischemic insult [19] or after the ischemic insult at the time of reperfusion [20]. In fact, post-ischemic treatment with RSR13 and dizocilpine also was shown not only to reduce cerebral infarct size, but also to significantly improve neurological outcome, compared to dizocilpine alone [20].

Summary

By enhancing the unloading of oxygen from hemoglobin, RSR13 represents a new therapeutic strategy to affect multiple clinical conditions in which tissue hypoxia plays a central role. RSR13 may improve the outcome of conditions caused by tissue hypoxia due to: 1) inadequate blood flow (regional or global), 2) insufficient oxygen carrying capacity (e.g., hemorrhage or dilutional anemia), 3) increased tissue oxygen demand unmatched by supply (e.g., myocardial ischemia), and/or 4) insufficient oxygen loading/unloading capacity of hemoglobin (e.g., hypothermia). Additional phase III clinical trials will evaluate the efficacy of RSR13 in these clinical indications.

References

1. Abraham DJ, Wireko FC, Randad RS, Poyart C, Kister J, Bohn B, Liard JF, Kunart MP. Allosteric modifiers of hemoglobin: 2-[4-[[(3,5-disubstituted anilino)carbonyl]methyl]phenoxy]- 2-methylpropionic acid derivatives that lower the oxygen affinity of hemoglobin in red cell suspensions, in whole blood, and in vivo in rats. Biochemistry 1992; 31:9141-9149.

(Continued on page 10)

RSR13

(Continued from page 9)

2. Teicher BA, Ara G, Emi Y, Kakeji Y, Ikebe M, Maehara Y, Buxton D. RSR13: effects on tumor oxygenation and response to therapy. Drug Development Research 1996; 38:1-11.
3. Kunert MP, Liard JF, Abraham, DJ, Lombard JH. Low affinity hemoglobin increases tissue po_2 and decreases arteriolar diameter and flow in the rat cremaster muscle. Microvascular Research 1996; 52:58-68.
4. Richardson RS, Jordan MC, Haseler LJ, Tagore KS, Wagner PD. Increased VO_{2max} with right shifted Hb-O_2 dissociation curve at a constant O_2 delivery in dog muscle in situ. J Appl Physiol 1998; 84(3): 995-1002.
5. Hall EJ. The oxygen effect and reoxygenation in radiobiology for the radiologist. 3rd edition, Philadelphia: Lipincott, 1988; 137-160.
6. Kunert MP, Liard JF, Abraham, DJ. RSR13, an allosteric effector of hemoglobin, increases systemic and iliac vascular resistance in rats. Am J Physiol 1996 271 (Heart Circ Physiol 40); H602-H613.
7. Pagel PS, Hettrick DA, Montomery MW, Kersten JR, Steffen RP, Warltier DC. 1997, RSR13, a synthetic modifier of hemoglobin-oxygen affinity, enhances recovery of stunned myocardium in dogs. J Pharm Exp Ther 1998; 285: 1-8.
8. Khandelwal SR, Randad RS, Lin PS, Schmidt-Ullrich R, Meng H, Pittman RN, Kontos HA, Abraham DJ. Enhanced oxygenation *in vivo* by allosteric inhibitors of hemoglobin saturation. Am. J. Physiol. 1993; 265(Heart Circ 34): H1450-H1453.
9. Grinberg OY, Miyake M, Hou H, Steffen R, Swartz H. The dose-dependent effect of RSR13, a synthetic allosteric modifier of hemoglobin, on physiological parameters and brain tissue oxygenation in rats. Submitted for publication. Oxygen Transport to Tissue XXII Edited by H Swartz New York, Plenum Press, 1999
10. Venitz J, Gerber M, Abraham D. Pharmacological effects of escalating IV doses of an allosteric hemoglobin (Hb) modifier, RSR13, in healthy volunteers. Pharm Res 1996; 13(9): S-115.
11. Kavanagh BD, Pearlman AD, Schmidt-Ullrich RK, Shaw EG, Dusenbery KE, Gerber MJ. Clinical experience with RSR13, a radiation-enhancing hemoglobin modifier, for recurrent and metastatic brain tumors. Tenth International Conference on Chemical Modifiers of Cancer Treatment 1998.
12. Kleinberg L, Grossman SA, Pantadosi S, Pearlman J, Engelman H, Lesser G, Ruffer J, Gerber M. Phase I trial to determin the safety and pharmacodynamics of RSR13, a novel radioenhancer, in newly diagnosed glioblastoma multiforme. J Clin Oncol 1999; 17: 2593-2603.
13. Woods JA, Storey CJ, Babcock EE, Malloy CR. Right-shifting the oxyhemoglobin dissociation curve with RSR13: effects on high-energy phosphates and myocardial recovery after

(Continued on page 11)

Oxygent

(Continued from page 6)

also demonstrated drug activity in terms of rapidly enhancing systemic and cerebral oxygenation status, reversing physiological "transfusion triggers" (i.e., the physiological parameters indicating the need for a blood transfusion),[3] and potentially reducing the need for allogeneic blood when combined with autologous blood collection. Phase 2a studies in cardiac surgery patients have demonstrated several potential benefits of using *Oxygent* during CPB. *Oxygent* enhanced both systemic and cerebral oxygenation status during bypass-induced anemia, and also decreased the incidence of intraoperative physiological transfusion triggers. With more aggressive pre-bypass autologous blood harvesting, the use of *Oxygent* demonstrated the potential to reduce the requirements for allogeneic blood transfusion.

Two multicenter, pivotal Phase 3 studies are currently underway. The first is a European study in noncardiac elective surgery and is targeted to enroll ~ 480 subjects at 30 centers in 8 countries. The second is a U.S. study involving cardiac surgery patients undergoing CABG procedures on CPB and is targeted to enroll ~ 600 subjects at 40 sites. Both studies will focus on demonstrating that A-ANH with *Oxygent* is a safe and effective method to reduce and eliminate the need for allogeneic blood in elective surgery patients.

References:

1. Keipert PE: Perfluorochemical emulsions - future alternatives to transfusion. In: Blood Substitutes: Principles, Methods, Products and Clinical Trials. Chang TMS (ed), Karger Landes Systems, Basel (1998): 127-156.
2. Habler OP, Kleen MS, Hutter JW, Podtschaske AH, Tiede M, Kemming GI, Welte MV, Corso CO, Batra S, Keipert PE, Faithfull NS, Messmer KF: Hemodilution and intravenous perflubron emulsion as an alternative to blood transfusion: effects on tissue oxygenation during profound hemodilution in anesthetized dogs. *Transfusion* 1998; 38: 145-155.
3. Spahn DR, van Bremt R, Theilmeier G, Reibold JP, Welte M, Heinzerling H, Birck KM, Keipert PE, Messmer K, and the European Perflubron Emulsion Study Group: Perflubron emulsion delays blood transfusions in orthopedic surgery. *Anesthesiology* 1999; 91: 1195-1208.

Meetings Minder

2000

Society for Technology in Anesthesia
January 12-15, 2000
Orlando, FL
http://gasnet.med.yale.edu/societies/sta/

3rd Biennial Difficult Airway Management
January 22-27, 2000
Vail, CO
http://cme.med.uth.tmc.edu/

Medicine Meets Virtual Reality 2000
January 27 - 30, 2000
Newport Beach, CA
http://www.amainc.com/MMVR/MMVR.html

2nd Virtual Congress in Anesthesiology
February 3-4, 2000
On-line
Http://www.reanimation.com

International Ergonomics Association 15th Triennial Congress in San Diego
July 30-August 4, 2000

6th Congress of Anesthesiology and Intensive Medicine
September 28—October 1, 2000
Kallithea-Halkidiki, Northern Greece
http://users.otenet.gr/~fkanak/foten5.htm

International Conference on Health Sciences Simulation
SanDiego, CA
Jan 23-27, 2000
www.scs.org

The 4th World Multiconference on Systemics, Cybernetics and I Informatics
SCI'2000
Orlando, FL
July 23-26, 2000
http://www.iiis.org

Ξ

(Continued from page 10)

low-flow ischemia. 1998; J Cardiovasc Pharmacol 31 (3): 359-363.

14. Weiss RG, Mejia MA, Kass DA, DiPaula AF, Becker LC, Gerstenblith G, Chacko VP. Preservation of canine myocardial high-energy phosphates during low-flow ischemia with modification of hemoglobin-oxygen affinity. J Clin Invest 1999; 103(5): 739-746.
15. Steffen RP. Effect of RSR13 on temperature dependent changes in hemoglobin oxygen affinity of human whole blood. Advances in Experimental Medicine and Biology, Vol. 454, Oxygen Transport to Tissue XX, Edited by Hudetz and Bruley, New York: Plenum Press, 1998; 653-661
16. Kilgore KS, Shwartz CF, Gallagher MA, Steffen, RP, Mosca RS, Bolling SF. RSR13, a synthetic allosteric modifier of hemoglobin, improves myocardial recovery following hypothermic cardiopulmonary bypass. Circulation 1999; 100(Suppl):II351-II356.
17. Watson JC, Doppenberg EMR, Bullock MR, Zauner A, Rice MR, Abraham D, Young, HF. Effects of the allosteric modification of hemoglobin on brain oxygen and infarct size in a feline model of stroke. Stroke 1997; 28: 1625-1631.
18. Grocott HP, Bart RD, Sheng HX, Miura Y, Steffen RP, Pealstein RD, Warner DS. Effect of a synthetic allosteric modifier of hemoglobin oxygen affinity on outcome from global cerebral ischemia in the rat. Stroke 1998; 29: 1650-1655.
19. Sarraf-Yazdi S, Sheng H, Grocott HP, Bart RD, Pearlstein RD, Steffen RP, Warner DS. Effects of RSR13, a synthetic allosteric modifier of hemoglobin, alone and in combination with dizocilpine, on outcome from transient focal cerebral ischemia in the rat. Brain Research 1999;826(2): 172-180.
20. Mackensen GB, Nellgård B, Sarraf-Yazdi S, Dexter SF, Steffen RP, Grocott HP, Warner DS. Post-ischemic RSR13 amplifies the effect of dizocilpine on outcome from focal cerebral ischemia in the rat. Brain Research 1999;853(1): 15-21.
21. Khandelwal SR, Lin PS, Hall CE, Truong QT, Lu J, Laurent JJ, Joshi GS, Abraham DJ, Schmidt-Ullrich RK. Increased radiation response of FSAII fibrosarcomas in C3H mice following administration of an allosteric effector of hemoglobin-oxygen affinity. Radiat Oncol Invest 1996; 4:51-59.
22. Rockwell S, Kelley M. RSR13, a synthetic allosteric modifier of hemoglobin, as an adjunct to radiotherapy: preliminary studies with EMT6 cells and tumors and normal tissues in mice. Radiat Oncol Invest 1998; 6:199-208.

Ξ