Challenges in Hyperbaric Oxygen Therapy and The PlasmaRx Opportunity: The Answer is Right Under Our Nose

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Introduction

Hyperbaric Medicine is a rapidly evolving discipline in health care. It is centered around the delivery of oxygen (O2) at increased pressure as treatment for a wide range of diseases and medical conditions (1). This treatment modality, known as Hyperbaric Oxygen Therapy (HBOT), is utilized alone and in combination with other treatments to which it is conceptually harmonious and complementary in practice.

It is the position of Evolution Hyperbarics, Inc. (EHI) that Hyperbaric Oxygen Therapy (HBOT) is underutilized as both a primary and adjunct treatment in the delivery of Health Care in the United States. Major contributors to this problem are myths and misconception that have developed because of insufficient scientific evidence to validate and support much of the use of this treatment modality. The inability of conventional HBOT systems to track and monitor the dose and dosage of oxygen (O2) delivered during treatment is a significant reason for a lack of acceptance, and until this challenge is overcome, it is unlikely that there will be significant scientific advancement in HBOT leading to increased approval by allopathic medicine.

The global mission at EHI is to promote the science of HBOT so that it will advance to the level of recognition, acceptance, and utilization that it deserves. However, EHI believes that to accomplish this mission, it is first necessary to insure the treatment dose and dosage of oxygen (O2) is delivered precisely as prescribed. Only then can valid scientific research and studies be performed that translate to the patient receiving optimal treatment. Fortunately, the PlasmaRx system developed by EHI has this capability and it is perhaps the most important improvement in HBOT technology in recent times.

PlasmaRx technology will help lead the HBOT field in fighting disease at the molecular level by enabling more precise and more effective treatment. At EHI, this new molecular-based method for designing and delivering treatment is termed “Precision HBOT” and we anticipate the principles underlying this approach will revolutionize the practice of Hyperbaric Medicine. The objective of EHI is to bridge PlasmaRx and Precision HBOT from invention to innovation through alignment with the Translational Medicine initiative of translating science-based findings from bench to bedside, from research to clinical outcomes,

within the community. It is realized that multi-disciplinary approaches and collaborative partnerships must be forged to accomplish the EHI mission and expedite further FDA clearance for use of HBOT as both primary and adjunct treatment.

The primary purpose of this document is to create an awareness of specific challenges inhibiting progress in Hyperbaric Oxygen Therapy (HBOT) and to introduce the novel PlasmaRx system as the opportunity to advance beyond these barriers. Advancement in HBOT is currently a slow process, but making PlasmaRx the technology platform from which to deploy this mission is an event that will accelerate advancement and catalyze a new paradigm in the delivery of HBOT.

This document is not a review of the subject of HBOT, but rather a compilation of topics selected to illustrate how challenges they present can be navigated and advanced through the opportunities presented by PlasmaRx and the Precision HBOT approach. Some topics were chosen to illustrate how a basic science such as organic chemistry can be applied to elucidate the biochemical pathways and molecular mechanisms that underlie the clinical outcomes observed for HBOT. Future documents will address specific diseases and conditions with focus on the challenges and opportunities they present. More in depth studies of biochemical pathways, chemical reactions, and mechanisms of action will also be presented over time. Furthermore, it is anticipated that special topics, recent developments, and current events will be covered in an ongoing fashion.

Overview of Challenges and Opportunity

In HBOT, the hyperbaric chamber and its operation are classified as a drug delivery system while oxygen (O2) is labelled a prescription drug and must therefore be used in accordance with U.S. Food and Drug Administration (FDA) guidelines and regulations. It will be seen in this document that HBOT is somewhat unique in that both the delivery of the drug (i.e. pressure) and the drug itself (i.e. O2) have therapeutic effects.

The application of HBOT in the treatment of disease and medical conditions is likely the most diverse of any prescription treatment modality, both in FDA-approved indications and off-label use. It involves treating a spectrum of disorders; from life-threatening cancer and neurological disorders such as stroke and traumatic brain injury (TBI), to cosmetic and Anti-aging/Rejuvenation Medicine. It is a treatment delivered in comfort and it is completely non-invasive with few adverse effects. After all, what is intuitively more therapeutic and healing than an environment of oxygen enrichment?

Despite the successful demonstration of efficacy and safety as an approved medical treatment, HBOT has not achieved widespread acceptance in mainstream medicine. Even though HBOT has more FDA approved indications than any other medical treatment, it remains marginalized in conventional medicine with often a perception of "treatment of last resort". This perception has unfortunately resulted in several myths and misconceptions about HBOT which are in part related to a lack of scientific evidence traditionally required to gain FDA clearance for use by a medical treatment. There is concern that there are too many far-reaching unsubstantiated claims of HBOT benefits and this has led to the FDA issuing a Consumer Update reflecting this agency's position of concern (2). As will be discussed later in this document, there is an inherent weakness to designing clinical studies involving HBOT due to limitations in creating a placebo control arm. Also, there has not previously been a system capable of accurately and routinely monitoring, tracking, and documenting the delivery of the dose and dosage of the treatment drug, oxygen (O2), as prescribed. This has been a critical challenge to the advancement of HBOT, for it is the delivery of a prescribed dose and dosage of the drug O2 that is the primary objective of this treatment modality. It is also generally considered a FDA requirement that the dose and dosage of a drug to be delivered as prescribed.

With all drug therapies, it is crucial to ensure the proper dose and dosage is precisely delivered so evaluation and comparison of the safety and effectiveness under different conditions can be performed. In some cases, it may be possible to grossly assess the effects and clinical outcomes of a drug treatment without accurately knowing the dose and dosage used, but it is not possible to determine the dose-response curve without knowing the precise amount delivered. Unfortunately, this has been the case for HBOT, and it has hindered the

ability to perform scientific research and studies that help determine the optimal conditions for treating different diseases and conditions. It has contributed to the inability to establish universally accepted standards of treatment care for HBOT patients.

Evolution Hyperbarics, Inc. (EHI) has developed a novel IT-based system called PlasmaRx which affords an opportunity to meet the challenge of delivering the O2 dose in HBOT. This breathing-gas management system was designed for the primary purpose of delivering the prescribed dose and dosage of O2 through monitoring, tracking and documenting the hyperbaric chamber %O2 during HBOT with precision. The PlasmaRx also tracks and monitors the chamber pressure during HBOT with far greater precision than standard HBOT systems.

The PlasmaRx system offers several innovative features that increase efficiency, reduce cost, and improve the patient experience. The method by which this system uses high pressure cylinder instead of liquid oxygen as the O2 source results in much less O2 waste and the chamber humidity and temperature are maintained at more comfortable levels. The noise during treatment is also much reduced compared to systems currently using liquid oxygen, helping to give the patient a more pleasant experience. High pressure oxygen cylinders are much more available on the global market and require substantially lower installation costs. Plasma Rx produces accurate, reliable, and reproducible data that are documented in a way that allows it to serve as an Electronic Health Record (EHR) system having the Health Information Technology (HIT) capabilities required for Medicare certification.

As mentioned above, the primary objective of HBOT is to deliver a prescribed dose and dosage of O2, but when this goal is accomplished using the PlasmaRx system, a quandary is created by not being able to adhere to the treatment pressure as prescribed. Typically, a prescription for HBOT is written as the delivery of 100% O2 at a specific pressure over a designated period to result a dose = %O2xatm/t. The PlasmaRx system has revealed that it is generally not possible to meet all three of these prescribed conditions during treatment.

The efficacy of HBOT is primarily attributed to reaching and maintaining blood plasma O2 level equivalent to that of inspiring 100% O2, or FiO2=1. However, PlasmaRx O2 sensing indicates that it is currently not possible to reach 100% O2 in

the chamber at the nasal location of the O2 sensor. But by following Boyle's Law, it is possible to increase the chamber pressure to achieve a ppO2 (O2 concentration) equivalent to that of 100% O2 at the original prescribed pressure, and because of Henry's Law, the ppO2 in plasma will reach the level that is considered of maximum benefit to the patient (i.e. the prescribed O2 dose and dosage).

In HBOT, the dose of O2 delivered in a treatment is a product of the %O2 times the pressure over time, or %atm/t. By raising the pressure to create the equivalent of 100% O2, the %atm increases, so the time, t, must decrease to keep the O2 dose from being greater than that prescribed. Therefore, the only way to reproduce all three parameters of %O2, pressure, and time is to begin chamber pressurization at the same %O2 with each treatment. Fortunately, the corrections in pressure and time are relatively small and the dose and dosage of O2 can always be delivered precisely as prescribed if using the PlasmaRx system to control, tract, monitor, and document the three parameters simultaneously. It can be seen that the interaction of parameters in HBOT are complex at best, so inherent limitations in the reproducibility of HBOT conditions exist. However, the PlasmaRX system minimizes the clinical impact of these limitations on patient treatment by insuring the prescribed dose and dosage of drug is delivered. This innovation alone affords the opportunity to advance the science and medicine of HBOT to standards previously unachievable.

Valid research and studies are necessary to assess clinical observations, validate claims, and explain clinical outcomes. There must be a scientific basis for designing treatment protocols, performing clinical studies, and FDA approval of indications. Also, Health Care providers need to know that they are ethically offering the best care available, for it is their responsibility to do the utmost to provide optimal patient care. There is also a need for providers, patients, and payers to be assured that treatments are delivered as prescribed. Accountability and integrity are paramount to the acceptance of HBOT.

PlasmaRx is also a technology solution for care and payment reform. Transformation of the U.S. Health Care system will bring more challenges, and PlasmaRx is designed to meet the HHS/CMS requirements for electronic health records (EHR) system certification, Health Information Exchange (HIE), meaningful

use, and quality measures reporting for value-based/merit-based payments. It is expected that PlasmaRx will have a significant impact on Hyperbaric Medicine by minimizing and streamlining the burden of transformation. Through informatics and analytics, PlasmaRx will provide guidance as HBOT facilities navigate through the transition from volume-based to value-based payments. And of course, this will translate into better re-imbursements for HBOT services.

The opportunity that PlasmaRx provides for advancement in HBOT is through Precision HBOT, and we envision PlasmaRx providing the technology platform from which a new paradigm in Hyperbaric Medicine will be launched. Precision HBOT is essential not only for insuring the delivery of the prescribed dose and dosage of O2, but also for the prevention of possible adverse effects such as O2 toxicity and barotrauma.

The mission at EHI is to establish a better understanding of treatment pathways, improve the design of protocols for studies and treatments, and progress the practice of Hyperbaric Medicine through Precision HBOT. Advancement in the "4Ps" of precision, pathways, protocols, and practice is the goal of this approach and each of these areas will be addressed.

Principles of HBOT

The use of HBOT is based primarily on the recognition that in certain illnesses, an increase in the amount of available oxygen (O2) promotes healing. To achieve an optimal disease-modifying plasma O2 concentration [O2 plasma] in the body using HBOT, an increase in both barometric pressure and the chamber % O2 are required. It is the ability to regulate both parameters over a specified period in a controlled environment that comprises HBOT. The therapeutic value of HBOT is therefore related to the three variables of chamber %O2, pressure, and time. It is important to note that in HBOT, pressure is an independent variable and [O2 plasma] is a dependent variable: the mechanical effects of pressure are independent of the [O2 plasma], while the [O2 plasma] maintained during treatment is dependent on the pressure at which it is delivered. The amount of time in treatment does not directly impact the other parameters. Time is simply

measured to determine when the prescribed dose and dosage of O2 has been reached at a set pressure and %O2.

The physical chemistry principles that determine the increase in O2 in the body during HBOT are described by Boyle's Law and Henry's Law for ideal gases (3). According to Boyle's Law, the product of pressure (P) times volume (V), for an ideal gas remains constant (i.e., P1V1=P2V2). Therefore, with constant hyperbaric chamber volume, increasing barometric pressure compresses the O2 gas, thus reducing its volume and effectively increasing its concentration. The increase in O2 concentration can be expressed as an increase in the partial pressure of O2 (ppO2), fraction of inspired O2 (FiO2), or %O2. Henry's Law states that the partial pressure of a gas such as O2 in solution (ppO2s) and the partial pressure of that gas in the air space (ppO2g) contacting the fluid surface become equal at equilibrium (i.e., ppO2s=ppO2g at equilibrium). Thus, the higher the ppO2 of inspired air, the more O2 dissolves in blood plasma and eventually all fluids and tissues in the body. However, equilibrium between ppO2 in the lungs and ppO2 in plasma is never realized since O2 is continuously consumed metabolically. There is therefore continuous diffusion of O2 from the lungs into the bloodstream.

Therapeutic Factors

Perhaps the most recognized use of HBOT in medicine is in the treatment of undersea divers for decompression sickness (DCS). This condition, commonly referred to as "the bends", can occur when a diver surfaces too quickly (4). It occurs because of rapid decompression allowing the formation of nitrogen gas bubbles that create embolism-related illness. The embolic events can cause serious injury and death, but HBOT is extremely effective for treating DCS and it is currently the treatment of choice for this condition. The primary factors involved in the therapeutic effect of HBOT in DCS are the physical dissolution of gas bubbles due of increased hydrostatic pressure, or recompression, and increased [O2 plasma] physically purging and replacing nitrogen in the body so that bubbles cannot reform. There is also promotion of healing of damaged tissues by the therapeutic action of O2.

Although O2 alleviates hypoxia and promotes tissue survival, the efficacy of HBOT in DCS appears to be related primarily to mechanical force rather than metabolic effects. In treating air embolism conditions where mechanical force is

primarily responsible for the therapeutic effect and the treatments are generally short-term, it is not as important to monitor and track the precise dose and dosage of O2 delivered. Indeed, with the generally emergent nature of DCS and arterial air embolism, it is recommended that relatively high barometric pressures be employed as the critical factor in initial treatment.

In the 1950s and 1960s, it was found that certain wounds and injuries healed faster with HBOT. Since that time, the application of HBOT in radiation injury and wound treatment has become part of the standard therapy for these conditions, and there are financial incentives supporting these indications. This success has helped promote interest in the use of HBOT as treatment for other medical conditions where increased oxygen might have therapeutic value. There are currently at least 15 medical indications for HBOT approved by the Undersea and Hyperbaric Medical Society (UHMS) and the FDA (5), and quite a few more medical indications are being investigated and considered for approval.

In all the medical treatments other than for DCS and arterial gas embolism, it is widely believed that the therapeutic benefits of HBOT are related primarily to the effects of O2 as a drug on metabolic pathways. It is therefore essential in these cases to insure the prescribed dose and dosage of O2 is delivered.

Oxygen as a Drug

The FDA generally considers oxygen a prescription drug. According to their regulations, O2 is considered a drug when the concentration (potency) inspired exceeds that which occurs naturally in air, and when it is intended for use in the "cure, mitigation, treatment, or prevention of disease or abnormal physiological process" under the FDA definition of a drug (6). Furthermore, the FDA regulations state that O2 to be used as a drug is considered a medical gas and must therefore meet the standards required to be labelled 'USP Oxygen', where USP stands for United States Pharmacopeia. As written by the FDA, "The USP specifications recommend that the potency of oxygen not be less than 99.0%." (7). It can be seen that there is a small margin of error allowed for the potency of USP O2.

As previously stated, O2 is perhaps the safest prescription drug currently available. It has the simplest molecular structure of any drug and it follows first

order pharmacokinetics. The absorption of O2 is determined by the laws of simple diffusion and its distribution requires no protein binding or active transport. There are no biological barriers to O2, for it can infiltrate all tissues and permeate all membranes. It requires no metabolic modification for activation or elimination, and unlike other drugs, it is consumed. It is a reactant in enzymatic and nonenzymatic reactions that modify both organic and inorganic compounds. Metabolic reactions requiring O2 generate a variety of oxidation products, including carbon dioxide, plus water.

Like other drug treatments, there is the potential for hyperbaric O2 interactions with medications, some of which may be clinically significant by either enhancing or inhibiting the actions and effects (8). These interactions occur through pharmacokinetic and pharmacodynamics mechanisms that are felt to be primarily O2-dependent, but may be due to O2 concentration plus hydrostatic pressure, or hydrostatic pressure alone in some cases.

It should be mentioned that there may be both desired and undesired interactions with illicit drugs, toxins, and nutritional supplements as well. At EHI, there is interest in using PlasmaRx and Precision HBOT to participate in investigations involving HBOT interactions with other types of pharmacotherapy. It is believed that the scientifically valid results of such investigations will be useful in the design of protocols to selectively target therapeutically desirable interactions and avoid undesirable ones.

Pressure bioactivity

The role of pressure in HBOT is generally recognized as simply part of a drug delivery system, whereby increased barometric pressure acts as a mechanical force that increases the concentration of O2 delivered to the patient by compression of the gas in the hyperbaric chamber. However, increased hydrostatic pressure is also known to exhibit biological activity and this property has been reviewed for biological systems in general (9), and for undersea diving, specifically (10).

An article was recently published in which the author specifically discusses the “acknowledged bioactivity of pressure” and he states “the bioactivity of increased atmospheric pressure is unknown to the clinical

hyperbaric medicine community, but well-documented in an extensive basic science literature." (11). Indeed, the physiological effects of pressure appear to be far reaching. The mechanisms of action for increased hydrostatic pressure are discussed later in this document.

Pressure and O2 bioactivity combined

Understanding how pressure and oxygen function together is critical to the advancement of Hyperbaric Medicine. Since both pressure and %O2 are likely important determinates to clinical outcomes, precise tracking and monitoring of both variables during HBOT is essential. The Plasma Rx system can supply these data so that scientific research and studies can be performed to elucidate how pressure and O2 function together. The results of these investigations can then aid in the design of the best protocols for delivering optimal patient treatments.

Since both pressure and %O2 may contribute to certain biological and clinical effects of HBOT, it is important to know if there are synergistic as well as additive effects with this combination of variables. Unfortunately, investigations tend to focus on the two variables separately. For example, there is evidence that increased pressure and %O2 separately increase the highly-ordered Exclusion Zone (EZ zone) structure of water significantly, while reduced pressure contracts the EZ zone (12). However, this study did not report experiments investigating the effect of increased pressure and %O2 combined, although it was suggested that there may exist an ordered water-mediated mechanism of action for HBOT.

There is also the possibility of antagonistic biological effects of pressure and O2 during HBOT, and separate pathways and mechanisms may be involved. However, this hypothesis can be tested only if precise monitoring and tracking of these variables is performed during studies.

Safety

Hyperbaric oxygen therapy is among the safest treatment methods available, with infrequent adverse events that are generally benign and reversible (13). By far, the two most common adverse events are middle ear barotrauma and claustrophobia. The importance of safety cannot be overemphasized given the current focus on harm and error prevention by the U.S. Health Care system. In

the U.S., death related to harm and error is estimated to be as high as 250,000 patients yearly, making this the third leading cause of death (14).

Precision

O2 dose and dosage

The dose of a drug is the amount administered as a single treatment, while the dosage is the total amount of a drug administered over all the treatments. This is analogous to driving a mile (dose) compared to the mileage (dosage) on the vehicle. The FDA generally requires the precise dose and dosage of a drug be delivered in all treatments for which it is prescribed. Furthermore, it is not possible to develop protocols and studies necessary to establish effective treatments if the delivered dose and dosage of a drug is not known. The FDA grants approval for indications using specific doses of a drug. Also, the drug dose and dosage being used should be known to ensure third party payers (health insurance) and patients (consumers) that they are getting what they are paying for.

Prior to PlasmaRx, there was not a system available to accurately continuously monitor and track %O2 precisely during HBOT. Therefore, it was not possible to determine the dose and dosage of O2 delivered for at least several reasons, it is likely that the O2 dose and dosage were generally below what was prescribed. For example, it is not possible to accurately use a set flushing time as a determinate for starting treatment since the time required for O2 flushing will vary from patient to patient based on their size (“displacement”), metabolic rate, respiratory rate, and tidal volume. Also, hyperbaric chambers generally operate using liquid oxygen as the USP O2 source, and even after warming by the evaporator and pressure regulators, the gas is substantially colder than the ambient temperature when it enters the hyperbaric chamber. The denser colder gas sinks to the bottom of the chamber and is vented out the back by a valve located near the bottom. All commercially available systems, other than the PlasmaRx system, measure %O2 in the vented gas outlet at or near the low point of the foot end of the chamber. This streaming gas creates a boundary between the warmer air above and the cooler O2 below that favors laminar flow of the

infused USP O2 rather than mixing. Therefore, the %O2 sensed in the vented gas in these systems is not that of the gas at the level of the patient's nose, so it has not been possible to accurately determine the length of time a chamber should be flushed with O2 to reach a %O2 inspired, or FiO2, acceptable to begin treatment. It should also be remembered that the purity (potency) of the USP O2 is merely "not less than 99%".

It has been demonstrated by EHI that replacing the air in the chamber with USP O2 prior to pressurization has generally been incomplete and highly variable, adding to the compelling argument that prior to the PlasmaRx system, there has generally been significant error in the dose and dosage of O2 delivered to the patient.

The PlasmaRX monitors O2 during treatment using a uniquely fabricated, highly sensitive O2 sensor that is mounted on a frame that causes little or no discomfort to the patient and strategically places the sensor adjacent to the nose at a minimal distance from a naris (nostril). In this location, the sensor is measuring O2 at a concentration essentially identical to that being inspired by the patient. This is clearly a significant improvement.

Pressure measurement

The precise measurement of pressure during treatment is as important as the precise determination of %O2. The use of analog gauges remains the standard method for measuring pressure in commercially available hyperbaric chambers, even though it is known that the gauges typically used on hyperbaric chambers are accurate only to within the range of (+) or (-) 2% of full scale. The analog gauges also require interpretation and they must be calibrated at least annually by a certified lab. Thus, analog pressure gauges have a significant margin of error and are considered antiquated by extremely accurate, reliable, and readily available digital gauges. The PlasmaRx incorporates a digital pressure gauge having a guaranteed error of no greater than 0.25% at any sampling speed. This improvement in the precision of measuring hyperbaric chamber pressure is another significant advancement in HBOT.

Pathways and mechanisms of action

Together, the fields of genomics, proteomics, and metabolomics investigate the continuum from what can be (genomics), the translation to what will be (proteomics), to what is (metabolomics) in cell physiology. These fields represent what is a seamless process from biological potential to expression, and anything that affects one area affects the other through a series of feed-back signals; you cannot change the one without changing the others. All pharmacotherapy therefore has a direct or indirect on gene expression. However, HBOT is the only treatment known to work therapeutically through direct action on each of these cellular domains, and all three may be targeted simultaneously during HBOT.

As previously stated, at EHI, it is believed that determination of the scientific basis for clinical outcomes is essential to realizing significant future advancements in HBOT. This includes the elucidation of biochemical pathways and molecular mechanisms of action. In the past, in vivo investigations on the mechanisms of action for O2 as a drug in HBOT were performed without knowing the precise dose or dosage of O2 delivered, so the results, and therefore the conclusions, of these previous studies may be unreliable and invalid. However, O2 dose and dosage can now be measured precisely using PlasmaRx, so investigations that generate accurate, reproducible, and valid data are possible.

There are multiple established and potential biochemical pathways, reaction mechanisms, and mechanisms of action involved in HBOT. However, this document is not a review of them. Only a select number of examples that are felt to be clinically significant and well-studied, scientifically verified or plausible, are presented and discussed here. Some examples are presented to demonstrate how basic organic chemistry reactions are involved in HBOT, and some examples are presented primarily to illustrate how and where PlasmaRX and the principles of Precision HBOT can readily be clinically applied.

Diatomic oxygen

The most stable, and by far the most abundant, molecular form of oxygen is dioxygen, O2. Because of its electron orbital structure, the electrons of O2 can shift readily to exist in several relatively stable resonance forms. More precisely,

the deformability of the electron cloud as described by quantum chemistry contributes to the molecular stability of O2. However, this easy flow of electron charge allows for polarization (dipole) to be readily induced by intermolecular interactions and contributes to the observed reactivity and bonding of O2 with many other molecules and atoms. The types of intermolecular bonds formed include covalent bonds, hydrogen bonding, and Van der Waals interaction.

Oxygen is the second most electronegative element on the periodic chart (fluorine is number one), which means it attracts electrons; it wants to react! This chemical property makes O2 the strongest free radical and electron scavenger in the body. It even scavenges electrons from its own free radical form, superoxide, to dismutate to hydrogen peroxide plus O2. Electronegativity is the property that accounts for the ability of O2 to serve as the final electron acceptor in the Electron Transport Chain of oxidative respiration energy production in mitochondria.

Diatomic oxygen is primarily consumed in mitochondria where it is converted to water and carbon dioxide in the final step of aerobic respiration energy production. The critical role that O2 serves in cellular reactions makes it equivalent to a nutrient (15), and perhaps nutriment, nourishment and food. Indeed, O2 is essential to everything in the human body; it is “The Breath of Life”!

Precision HBOT and Precision Medicine

The success of HBOT has been remarkable despite the crude estimates of O2 dose and dosage delivered. However, it is now possible to deliver the dose and dosage of O2 precisely as prescribed by implementation of PlasmaRx. Using this advancement, scientific research and studies can be designed to determine the optimal treatment conditions required to target specific metabolic pathways and molecular mechanisms with precision and effectiveness. Precision HBOT as a molecular-based approach to treatment was created to provide more precise and more effective treatments, and the scientific principles behind this method are expected to elevate Hyperbaric Medicine to a new level.

To explain the observed benefits of HBOT requires identification of the biochemical pathways affected by treatments and elucidation of the precise molecular mechanisms of action. Metabolomics is a field of science that employs

powerful analytical methods of chemistry to identify relatively small organic molecules that are products of metabolism (16). Together, these products comprise the body of metabolism, or "-ome", that reflects both normal and abnormal processes in an individual. Through metabolomic studies, molecular biomarkers unique to a specific disease process can be identified for monitoring progression or regression, response to treatment, and perhaps treatment safety. Biomarkers may also reveal pathways by which to design new targeted treatments.

Pharmacogenomics is the study of how the genome affect a person's response to drugs with the hope of developing safe, effective medications and doses that are tailored to a patient's genetic makeup (17). It is another field of science that is expected to contribute to the evolution of Precision HBOT. Although there are currently no known pharmacogenomics data or studies for O2 (18), it is anticipated that this will quickly change once there is widespread implementation of the PlasmaRx system. Perhaps epigenetic effects of HBOT will be beneficial to the patient's response to other drugs or otherwise affect the genome in a beneficial manner.

Precision Medicine is an emerging approach in the U.S. Health Care system that is aimed at targeting prevention and disease treatment to the unique genetics and ecosystem of each person so that the best outcomes can be realized (19). It is based on determining the biomarkers and genomic markers for a given disease to identify a molecular abnormality at which to target a selective treatment and monitor the response. As such, it is more individualized than personalized medicine since treatments are designed to target disease based on specific characteristics that are shared by individuals similarly affected, not just a specific person.

At EHI, it is believed that Precision Medicine and Precision HBOT are fully aligned, compatible and complementary. For only through research such as metabolomics and pharmacogenomics can the precise optimal conditions for targeted treatments be determined and Precision HBOT evolve and be implemented. The Precision Medicine Initiative announced by president Obama in early 2015 has the goal of "targeting the right treatments, to the right patients, at the right time" (20).

Mechanisms of Action for O2 in HBOT

The number of metabolic pathways requiring O2 and the multiple mechanisms of action associated with them illustrate the fact that O2 is the most metabolically diverse substance in by the human body. It is also the most important molecule to human survival based on time. This point is exemplified by the "Rule of Threes" taught in survival training: you have three minutes to find air (oxygen), you have three hours to find water, and you have three days to find food. The use of HBOT delivers markedly increased amounts of O2 to all parts of the body with treatment of all susceptible conditions simultaneously. It is a systemic treatment that is neither selective nor specific and there are few adverse effects associated with its use, none of which are life-threatening. However, many of the pharmacologic and most of the pressure-related mechanisms of action for O2 in HBOT remain poorly understood. In this section, it will be attempted to provide specific examples of known mechanisms of action and to suggest some areas of where further investigation may be of significant benefit.

Oxidoreductase reactions

One of the oxygen-dependent pathways most affected by HBOT are those requiring a cytochrome oxygenase step. These enzymes possess a heme-like structure that chelates a transition metal such as iron, copper, or manganese. The metals have a strong affinity for binding O2, and once bound, the O2 becomes highly reactive and the subsequent reactions consume O2 as a reactant. These are known broadly as "oxidoreductase" enzymes (21), and belonging to this class are members of the cytochrome P450 system. Oxidoreductase enzymes perform many reduction-oxidation (redox) reactions including those involved in the synthesis of steroids and steroidal hormones such as vitamin D and the sex hormones. They are also involved in the synthesis and degradation of heme, multiple neurotransmitters, collagen, and cartilage to name a few. Certain steps in drug metabolism, detoxification, and energy production pathways also require these enzymes.

It is recognized that the availability of O2 is potentially the rate-limiting step in many O2-dependent enzymatic reactions in biological systems. The kinetics of these reactions is sometimes controlled by the availability of both substrate and O2 as a reactant. Therefore, in cases where the concentration of O2

is rate-limiting, the use of HBOT to deliver increased O2 would be expected to accelerate the reaction. The result could be clinically desirable or undesirable, but in either case, the precise dose and dosage of O2 delivered in treatments must be known if one wishes to target and control the reactions. This, of course, can only be achieved using PlasmaRx, and it is conceivable that applying Precision HBOT principles with this technology will lead to the development of treatment protocols that selectively target the desired oxidoreductase systems and avoid the undesirable ones to achieve beneficial clinical outcomes.

Proteomics

Proteomics is the study of proteins, primarily their structure and function, on a large scale as the proteome (22). The proteome is considered the collective body of proteins and the influence of the genome on their production and modification. This field of study is essential to the understanding of the translation from the genetic message to the expression of products of metabolism.

It appears the effects of HBOT on protein structure and function have been largely ignored, but this area potentially holds the opportunity to greatly expand the use and understanding of HBOT. Although increased concentration of O2 is likely the primary factor involved with these effects, the role of increased hydrostatic pressure should not be ignored, nor should the potential additive or synergistic effects of these variables in combination be overlooked.

Most known mechanisms of action for O2 in HBOT involve its consumption in enzymatic reactions, but it is plausible that O2 also binds to enzymes in a manner that results in the regulation of enzymatic activity through mechanisms that do not consume O2. For example, O2 may bind to an enzyme at a site other than the active site and regulate the enzymatic activity through a change in the active site conformation. This form of functional control of enzymes through a transformation in protein shape is known as allostery. More generally stated, allosteric regulation enables the activity of one site on a protein to modulate function at another spatially distinct site (23) and allosteric communication is of fundamental importance to nearly every biological process (24). There may be multiple allosteric binding sites and multiple effector molecules for a given enzyme or enzyme system. A molecule that has an allosteric

effect is known as an “effector” and it can be an enzyme reactant, substrate, or product or it may merely bind to the enzyme, having no direct participation in the enzyme-catalyzed reaction.

Even though hemoglobin (hgb) is not a catalytic enzyme, it is a model for how O2 has an allosteric-like effect on a protein. When the first molecule of O2 binds to hgb, an allosteric effect occurs that increases the affinity for each subsequent O2 binding to each of four subunits until all four sites are saturated (25). When CO2 is generated in tissues and released into the blood stream, it is converted into carbonic acid and protons are subsequently released. These protons bind to hgb and reduce its affinity for O2. This effect is known as the Bohr Effect. When inspired O2 binds to hgb, the binding of CO2 to the N-terminal end as a carbamate is reversed and the CO2 is released into the lung as an exchange process.

It is reasonable to assume that there are multiple enzymes and enzyme systems that are allosterically regulated by O2 during HBOT, when very highO2 concentrations likely lead to saturation of all possible binding sites, even those having low affinity for O2. Indeed, it is likely that there are sites on enzymes that do not bind O2 at all with normal physiologic O2 concentrations, but do bind O2 with conditions typical for HBOT.

Blood plasma is considered the most informative proteome from a medical viewpoint, since the human plasma proteome contains most, if not all, human proteins (26). This diagnostic potential has led to a surge in investigations into the discovery of disease markers by a wide range of proteomics strategies. It is believed that these strategies will be immense contributions to advancements in HBOT when coupled with PlasmaRx and Precision HBOT.

It is plausible that during HBOT, O2 binds to and effectively saturates many plasma proteins such as albumin and other carrier proteins, enzymes, lipoproteins, and even antibodies. Therefore, with enough pressure and O2, all human proteins may be O2-carrier proteins. This phenomenon would be expected to result in enhanced delivery of more O2 to distal regions of the body where desaturation will occur as the O2 concentration (oxygen tension) declines. Perhaps this phenomenon contributes to the effectiveness of HBOT. It must also be considered that allosteric effects of HBOT on plasma proteins might change

the capacity of plasma proteins that function as carriers of other substances, such as hormones, nutrients, drugs, and inactive metabolites. Also, hydrostatic pressure and O2 concentration may regulate enzymatic activity and even induce enzymatic activity in otherwise inactive proteins. There is growing evidence that certain plasma proteins previously considered biologically inactive are enzymes under specific conditions. For example, there is evidence that suggests all antibodies have an inherent ability to generate hydrogen peroxide from O2, which aligns recognition and killing immune function within the same molecule (27).

There is also the possibility that O2 saturation modifies the usual function of plasma proteins. For example, albumin is a carrier protein for numerous drugs and metabolites including certain hormones, and the binding of these may change during HBOT in a way that significantly effects the pharmacokinetics and pharmacodynamics of certain drugs as well as the equivalent behavioral characteristics of transported metabolites.

Many O2-dependent biochemical pathways are enhanced by HBOT conditions, but some are inhibited. For example, studies suggest cyclooxygenase, tyrosine hydroxylase, and phenylalanine hydroxylase are inhibited during HBOT (28). Interestingly, these enzymes catalyze the addition of O2 to their reaction substrate as the initial step in the synthesis of important signal molecules. Although it is possible that O2 is acting through allosterism in these and some other, it may instead be acting as a traditional enzyme inhibitor. It is also plausible that the mechanism of action for these examples, as well as for many other effects of hyperbaric O2 on the proteome, may be unrelated to enzyme interaction of any type, but rather related to epigenetic pathways which will be discussed in the following two subsections.

It is possible for enzyme inhibition by hyperbaric O2 to occurs through each of three known mechanisms. When the concentration of O2 at the catalytic site exceeds a certain limit, simple steric effects can lead to “competitive inhibition” whereby there is direct interference with substrate binding, or “uncompetitive inhibition” where binding elsewhere in the active site disrupts substrate-enzyme complex formation. The third type is “noncompetitive inhibition” and this is inhibition of enzyme activity through binding at a site other

than the catalytic site and has an allosteric effect of changing the enzyme conformation.

A recent area of interest in allosteric enzyme regulation is with morpheeins. Morpheeins are homo-oligomeric proteins where the oligomers dissociate, the dissociated units change conformation, and the altered conformational state units reassociate to a distinctly different oligomer, both structurally and functionally (29). Loss in the ability of a morpheein to participate in this structural dynamic can result in a conformational disease such as in a form of porphyria where there is a genetic mutation to the porphobilinogen synthase gene (30). Interestingly, porphobilinogen synthase and O2 are both required in the synthesis of porphyrins including heme.

Targeting epigenetic pathways is also a strategic approach to treatments that ultimately rely on regulating the proteome, since proteins are the major products encoded in the genes. It is also plausible that post-transcriptional processes including the modification, packaging and distribution of proteins are also affected by HBOT and can be treatment targets as well. Indeed, HBOT may affect the proteome more than any other single treatment. It is believed that HBOT may also become an important method for proteomic investigations!

At EHI, it is believed that elucidation of the effects of HBOT on protein structure and function, and therefore the proteome is crucial to understanding some of the clinical effects of HBOT. It is expected that having this knowledge will resolve some of the controversy surrounding HBOT, but it will require delivery of the precise O2 dose and dosing to be accomplished. It is also likely that precise dose and dosage will be required to selectively target certain desired protein regulation. The field of proteomics is yet another area of science to further investigate using PlasmaRX and the principles of Precision HBOT.

Oxidative Stress

“Oxidative stress” and “free radicals” are not synonymous terms, they are not the same! There appears to be a common misconception and confusion over the difference in these terms and a clear delineation between them must be made to fully grasp and understand their physiologic roles. Not all free radical production creates oxidative stress.

Oxidative stress is a physiologic condition associated with an imbalance in the production of highly reactive free radicals and the cell's ability to neutralize them using anti-oxidants. Therefore, oxidative stress is not proportional to the production of free radicals which is an unavoidable, inherent, and necessary consequence of normal aerobic metabolic processes. In fact, a low-level range of free radicals is essential for signaling in certain physiological pathways and excess production beyond a certain threshold is normally countered by protective antioxidant systems. Oxidative stress only occurs when the production of free radicals becomes too great and overwhelms the protective antioxidant systems of an organism to allow oxidation reactions with critical biomolecules such as proteins and DNA. These reactions are potentially damaging, cumulative, and toxic, and therefore stressful to the organism.

In chemistry, radicals are molecules having an atom with an unpaired electron in the outer electron orbit of the electron shell surrounding the nucleus. This electron is "free" from pairing, and with few exceptions, it is "free" to be shared with other molecules or itself in bond formation. The unpaired electron is looking to find a partner for stability, so it is generally an unstable molecular species, and therefore highly reactive. Free radicals are frequently referred to as "reactive species".

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are major classes of free radicals produced in the human body and they play a critical role in cell physiology. Under normal conditions, a small number of these molecules escape the metabolic pathways in which they are involved, but very efficient antioxidant systems exist to capture (neutralize) the radical escapees before they can cause trouble. However, when abnormally high amounts of free radicals escape due to failure of metabolic controls, antioxidant systems can become overwhelmed as mentioned, and the excess radicals are then able to initiate oxidation reactions that cause cellular damage. This oxidative stress not only can injure a cell, but it also serves to maintain homeostasis by constantly messaging the cell as to the status of metabolic pathways and the condition of the metabolic machinery so that appropriate repairs are made or apoptosis (programed cell death) is initiated.

Following the free radical theory of aging proposed in 1956 (31), and prior to the understanding of the essential biological roles of free radicals, it became dogma that free radicals are generally abnormal and unwanted byproducts of metabolism that damage critical cellular components such as proteins and DNA, and it is the accumulation of this type of cellular damage that primarily results in aging. Even though it is now known that the generation of free radicals is generally well controlled and that they serve as signal molecules in the transduction of cellular messages, this misconception is only slowly being dispelled, as one can readily see by the existence of a lucrative industry based on marketing products to combat aging through antioxidants therapies. Without the proper number of free radicals and oxidative stress, our cells become dysfunctional, leading to disease and possibly death.

There is without a doubt an adaptive advantage to the dilemma of the formation of free radicals as useful signal molecules versus damaging oxidative stress. By merely invoking arguments of evolutionary bioenergetics, cellular metabolism would not have been allowed to evolved to the use of O2 metabolism as a primary source of energy production unless there was a significant net benefit. The adaptive pressure to conserve energy is the competitive driving force for all of life! The electronegativity, and therefore inherent reactivity, is the very quality of O2 that renders it the most efficient molecule for energy production, but at the expense of forming potentially damaging free radicals. However, even the formation of these potentially harmful molecular species has been channeled into positive effects.

It has been stated that, “Principle mechanisms of HBO2 are based on intracellular generation of reactive species of oxygen and nitrogen.” (32).

Reactive oxygen species

It is well accepted that breathing greater than 1 ATA O2 will increase production of ROS proportional to the O2 concentration/tension (33). However, cellular antioxidants adequately prevent oxidative stress damage during HBOT.

As previously mentioned, the formation of ROS is a consequence of the inherent electronegativity of O2 and its conversion to radicals requires no enzyme or other catalyst to initiate, although transition metals, especially iron, and

transition metals complexed with porphyrins readily catalyze ROS generation from O2.

Even though oxidative stress due to the production of ROS is a concern, ROS has functions other than to activate defense mechanisms to protect against ROS damage. An excellent example of the importance of ROS to proper cellular function is found in the mitochondria of eukaryotic cells. As the most electronegative molecule in the body, O2 is used as the terminal electron acceptor to complete the Electron Transport Chain (ETC) for aerobic energy production. The generation of ROS can occur during this final step of the ETC where an electron is transferred to O2 to form superoxide, the radical anion form of O2. Some electrons also escape during other reactions along the ETC, but O2 is present to scavenge the leaking electrons which also generates superoxide. Although superoxide is highly reactive, it is quickly scavenged by mostly endogenous defense antioxidants and eventually converted to non-reactive species. There are also multiple exogenous antioxidants involved in savaging and quenching free radicals, including vitamin E, vitamin A and vitamin C.

Superoxide is first converted to another ROS, hydrogen peroxide (H2O2), and O2 the enzyme, superoxide dismutase (SOD). Then, to protect the cell from oxidation by H2O2, hydrogen peroxide is converted to H2O+O2 by the enzyme catalase. The peroxidase family of enzymes also aids in the conversion of H2O2 to H2O+O2 with glutathione peroxidase also converting lipid peroxides (ROOH) to the corresponding lipid alcohol (ROH)+H2O to help preserve membrane integrity. Another ROS that can form is the symmetric dissociation of H2O2 to two hydroxyl radicals (2OH). The hydroxyl radical is the most unstable and therefore the reactive and damaging ROS. It even reacts with chloride ion to generate hypochlorous acid (HOCL) that is sodium hypochlorite (NaOCL) at physiological pH, the active ingredient in bleach.

However, antioxidant protection is under certain oxidation conditions overwhelmed to allow for cell damage by ROS. Although this oxidative stress can lead to cellular injury, it is also the initial signal for the need to repair faulty, worn-out parts (protein subunits) before the need to replace an entire engine (complex) or the entire machine (mitochondrion), or even the entire cell, through apoptosis. In this case, superoxide formation is the first step in signal transduction to

transcription factors that lead to transcription of the gene for the required protein(s) or apoptosis. This is also an example to demonstrate how the metabolome, genome, and proteome work together in concert.

It has been proposed that another ROS signaling pathway is through oxygen radicals serving as messengers in O2-dependent gene expression. This pathway is not only relevant to ROS messaging, but also to mechanisms of action by which HBOT has epigenetic effects. Therefore, it is felt that this pathway will be discussed in detail in the "Epigenetics" section below.

Nitric oxide

Nitric oxide is a ubiquitous cellular signaling molecule in the human body. It is involved in the modulation of numerous critical processes including vascular tone, platelet activity, airway tone, peristalsis, angiogenesis, insulin secretion, and neural development. It is primarily formed by the nitrous oxide synthases (NOSs), a family of enzymes catalyzing the reaction of O2 with the guanidine moiety of the amino acid arginine. The NOSs also catalyze "leak" and other side reactions such as the formation of superoxide from O2. There is a general increase in the production of NO with HBOT (32) and in the treatment of several medical conditions including wound healing (34).

Three isozymes of NOS have been identified that modulate different physiological processes and are associated with different cell types. These isozymes are labelled epithelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). The inducible isoform is involved in the immune response where NO, being a free radical, appears to serve primarily as an oxidizing agent. The binding affinity for O2 is different for each of these isozymes, so it is plausible that they can be selectively targeted using different concentrations of O2 in HBOT. The small difference in the O2 affinity of these isozymes offers a narrow therapeutic window which translates into a requirement for very precise treatment conditions. Therefore, it will be necessary to implement PlasmaRx in the development and delivery of these treatments.

Epigenetics and gene therapy

Epigenetics is the study of the influence environmental factors have on gene function and regulation. The epigenetic effects of HBOT are well recognized

and it has been stated that HBOT is “the oldest, most enduring, and most effective gene therapy” (11). It has been reported that as many as 8101 genes are either up-regulated or down-regulated at 24 hours following a single HBOT exposure. The up-regulated genes were primarily for growth and repair, and anti-inflammatory products, and the down-regulated genes were primarily for proinflammatory and apoptotic products (35). However, the associated biochemical pathways and mechanisms of action are only beginning to emerge and some of these have already been presented in this section.

Most O2 epigenetic effects appear to be transduced from its action on transcription factors, either directly or indirectly. For example, HBOT modulates the expression of hypoxia-induced factor-1 (HIF-1) (32), a protein considered the main regulator of multiple physiologically important O2-sensitive genes (36). Perhaps the most compelling evidence for the importance of epigenetic pathways and the link to ROS in HBOT biological activity is in the regulation of O2modulated genes.

Changes in ambient O2 concentration need to be sensed by cells for long-term adaptation through the regulation of gene expression. It has been suggested that cellular O2-sensing for long-term adaptation of cellular functions relies on ROS (37). Since the production of ROS is known to be proportional to the ambient O2 concentration, they are ideal candidates as messengers in the modification of transcription factors modulating gene activity in response to the O2 concentration. There is evidence suggesting the O2 sensor is a heme protein like the heme-containing NADPH oxidase found in mammalian neutrophils. This O2-dependent enzyme produces superoxide which is subsequently dismutated to H2O2. The H2O2 is then involved in an iron-dependent perinuclear Fenton reaction to form hydroxyl radicals that modify transcription factors such as hypoxia-induced factor-1 (HIF-1), vascular-endothelial growth factor (VEGF), and activator protein-1 (AP-1). It is believed that the mechanism by which the hydroxyl radicals affect change is through oxidation of disulfide bonds present in the transcription factor proteins. This creates a redox system for regulation of O2modulated genes which responds to low levels of O2 by maintaining more reduced form, and at higher O2 concentration, maintaining more oxidized form. Both forms can be active in gene expression depending on the transcription factor involved.

There are other mechanisms by which HBOT appears to exert bioactivity through epigenetic pathways, and these include pressure as a participating influential parameter (11). Due to the attention, the epigenetic pathways deserve and require to understand, this topic will be elucidated in subsequent EHI documents. However, it can be seen through the sensitive and tightly controlled O2-modulated genes model that it is again crucial to determine and measure the precise dose and dosage of O2 delivered during HBOT, as can only be achieved using PlasmaRx. Also, the molecular mechanism of action proposed for this pathway is a great example of the need to follow Precision HBOT principles in the design of treatment protocols.

Inflammation and immunity pathways

Inflammation and immunity are critical to health promotion, disease prevention, and the healing process. They are strongly linked and interrelated, with considerable overlap in their response, function, and actions and they are synergistic when combatting a common enemy. For example, inflammation is one of the earliest responses to infection and ushers in the immune response to work together. Both immunity and inflammation are signaled by chemical messengers in response to an invader or insult to the body. Although the specific cell types vary, leukocytes are recruited in both processes. Both systems also have an acute phase, as well as a potential chronic phase which can become a disease.

It has been demonstrated that both and inflammation and immunity are modulated by HBOT. The effects on inflammation are evident by FDA approval for use in the treatment of multiple diseases and conditions associated with inflammatory component (5). However, there is considerable evidence that HBOT is beneficial in many other inflammatory disorders, but further investigations are needed to be granted clearance for use in these.

Depending on the pressure used and the experimental model, HBOT has been shown to stimulate and depress immunity (38). However, treatment generally has a clinically beneficial effect, but it is recognized that much more investigation into the effects of HBOT on immunity is necessary to explain the beneficial effects in infections, disorders of the immune system, and transplantation.

Given the ability of HBOT to both stimulate and suppress inflammation and immunity, it is plausible that these are examples of HBOT merely providing conditions that enhance the performance of these systems in the specific disorder they are involved. Multiple biological pathways and mechanisms are involved in the critical role that O2 has in inflammation and immunity, and these will be discussed in detail in future EHI documents.

For the purposes of this document, it is felt to be sufficient to merely state that the mechanisms of action of O2 in inflammation and immunity are in many cases well understood and appreciated. Importantly, it is believed by EHI that the ability to deliver the precise dose and dosage of O2 during HBOT is critical to designing optimal treatment protocols that target specific molecular mechanisms involved in the function of these pathways. Inflammation and immunity clearly afford excellent opportunities to demonstrate Precision Medicine through PlasmaRx and Precision HBOT, and these subjects will be addressed.

Stem Cells

It has been reported that HBOT stimulates stem/progenitor cell (SPC) mobilization without inducing leukocytosis and it was determined that this phenomenon is dependent on NO production by bone marrow eNOS (39). Also, data from this investigation suggest that the increase in SPC availability induced by HBOT is due to enhanced bone marrow production as well as mobilization, and this response is observed for healthy as well as diseased individuals with certain disorders. It is plausible that HBOT also stimulates increased production and mobilization of stem cells that promote the healing of other conditions and diseases. The potential clinical benefits of this phenomenon are enormous, but without knowing the relationship of this action to specific treatment conditions, it is simply not possible to fully develop this concept.

Using the PlasmaRx system and Precision HBOT principles, it may be possible to develop valid standard methods for the clinical application of this action to cell therapy. For example, HBOT may augment or even replace the need for harvesting SPCs. It is the intention of EHI to be involved in investigations on these and other potential advancements in cell therapy with HBOT.

Mechanisms of action for pressure

It is equally important to elucidate the mechanisms by which increased pressure exerts biological activity in HBOT. Even though investigations on piezoelectric effects and possibly other pressure-dependent pathways will generally employ methods other than those for O2 pharmacology, precise measurement of chamber pressure throughout treatment is required to generate valid data. Again, the Plasma Rx system will meet the challenge.

Mechanotransduction is the conversion of a mechanical force, such as pressure on a cell, to an electrochemical signal which can result in a variety of cellular responses (40), including those of sensory cells associated with touch, hearing and balance. The human body also has specialized pressure sensors called baroreceptors strategically located throughout the cardiovascular system to regulate hemodynamics and includes such well-known baroreceptors as those located in the aorta and carotid arteries. Although a variety of signal pathways may be involved, the basic mechanism is stimulation of a mechanoreceptor leading to opening of a gated channel in the cell membrane. This results in the flow of ions across the membrane that changes the membrane potential, sending an electrical or chemical signal.

Piezoelectricity is another pathway by which hydrostatic pressure can exert a biological effect. The precise molecular mechanisms by which pressure exerts cellular piezoelectric effects is unknown, but it has been proposed that it involves alteration in cell and mitochondrial membranes (41). When subjected to increased hydrostatic pressure, these lipid-bilayer membranes exhibit liquid crystal characteristics which may create a separation of charge across the membrane, and this electrical change leads to a chemical signal.

The piezoelectric and the mechanotransduction effects are both felt to convert mechanical force into chemical signals and there is evidence for the action of increased hydrostatic pressure on cell membranes having epigenetic effects (42). Increased hydrostatic pressure may even have been essential to the creation of early life in the “Primordial Sea”. This theory is readily appreciated when one considers the presence of life in hydrothermal vents at great depths (>5000 feet) in the ocean.

Physicochemical effects on proteins is another pathway by which hydrostatic pressure creates biological effects that may be consequential. Effects of hyperbaric conditions on proteins have been studied and described (43). Intramolecular interactions such as protein conformation, folding, and allosterism, as well as intermolecular interactions such as dipole, ionic, Van der Waals' forces and hydrogen bonding are all subject to pressure effects.

The use of PlasmaRx and Precision HBOT are expected to contribute to the elucidation of the mechanisms of action for increased hydrostatic pressure during HBOT since precise and reproducible measurements of pressure are required in such investigations.

Protocols

Hyperbaric oxygen has been shown to be therapeutic in a wide range of health problems, including some that have been poorly responsive, or unresponsive, to other treatments. In fact, it has become first-line treatment for certain disorders such as decompression sickness, carbon monoxide poison, and arterial gas embolism. Although most approved indications employ HBOT as a "last resort" treatment, it is becoming increasingly clear among Hyperbaric Medicine professionals that HBOT is underutilized as a primary treatment or a treatment early in disease. There are also many medical conditions where HBOT appears to be extremely effective, but for which FDA approval has not been granted. Thus, it is common practice for HBOT to be used off-label, frequently to treat diseases for which there is no effective treatment otherwise. It has been stated that HBOT is "so effective, it is a threat to Medicine…" (44).

Unfortunately, well-researched, science-based protocols for the delivery of HBOT are few and this has led to a lack of acceptance of HBOT by many health care providers and other professionals. However, the unique technical complexity of HBOT simply does not allow for standard Randomized Controlled Trials (RTCs) to be performed as with other drugs. This inherent limitation has led to difficulty in acceptance by evidence-based medicine which relies on primarily blinded RTCs results as the measure of strength of evidence for a treatment.

As previously discussed, increased pressure alone has biological activity and increased pressure increases the ppO2 of any gas containing O2. Therefore, any increase in pressure delivers O2 as a drug, even when ambient air is breathed (i.e. the ppO2 of inspired air increases with increasing pressure). It must be realized that any attempt to consider a sham or placebo control arm in a study is futile since a patient will generally detect whether there is an increase in pressure or not. However, there is report of a method of patient blinding using a minimal air compression technique which may be an effective tool for patients enrolled in HBOT trials (45).

Perhaps the leading obstacle to advancement in HBOT is the wide range of conditions (i.e. pressure, %O2, time) used for treatments. There appears to be the assumption that the %O2 inspired by the patient is “close enough”, but this has been shown previously in this document to not be valid without appropriate monitoring and tracking as with the PlasmaRx system. Of equal or greater concern is the wide range of the treatment pressure and time variables. Different studies frequently report using different chamber pressure and different periods of treatment time, and the variability is present not only between different treated conditions, but also within a single condition. This of course makes it not possible to compare individual or study results, which leads to uncertainty and confusion as to the safest and most effective treatment for a given medical condition.

The challenge of knowing the best pressure and time parameters for a given treatment has been addressed with the conclusion that there is currently insufficient evidence available to recommend one treatment over another (46). Therefore, for treatments such as wound healing, choosing one of several protocols that were developed based on clinical observed and experience. These clinical protocols appear to be considered generally clinically equivalent and effective based primarily on consensus, but they may both be merely adequate at best. However, this is an opportunity to establish a standard of care that is based on scientific evidence as well as clinical observation. With wide-spread acceptance and implementation of PlasmaRX and Precision HBOT principles, it will be possible to perform comparative studies where there is tight control over the treatment variables and thus develop protocols based on valid scientific evidence. Although conventional RCTs may not be possible to perform, it will be possible to generate

sufficient accurate, consistent, reliable and reproducible data on which to establish standards of care and practice evidence-based medicine. And fortunately, the FDA has developed new pathways for drug use clearance/approval that do not depend as heavily on RTC evidence (47). For HBOT, this has been the case all along.

The FDA clearance for use (equivalent to indications approval) of HBOT has not previously been based primarily on clinical outcomes that include accurate, reliable, and reproducible data as in RTCs. This lack of supporting data is inconsistent and noncompliant with the FDA standards generally required to be met for use of a prescription drug or treatment. And indications for a drug are usually granted by the FDA based on the use of specific dose and dosage values. The clearance process for HBOT use is an exception to these rules, with the FDA relying on the Undersea and Hyperbaric Medical Society (UHMS) for guidance on the safety and efficacy of HBOT for specific diseases and conditions (48). The Hyperbaric Oxygen Therapy Committee of the UHMS evaluates valid scientific evidence regarding the safety and effectiveness of HBOT for clinical indications to determine which merit approval. Although the UHMS is recognized by the FDA as the leading authority on HBOT efficacy and safety, the validity and appropriateness of this assumption has been challenged (49).

There is a new initiative in designing Observational Studies as part of drug evaluation during the approval process (50). These studies primarily of the cohort and case-control type. As an investigational drug progresses through research and development phases, there is a shift from “Does the drug work?” to “Does the drug work in the real world?”. Ideally, data collection for Observational Studies should be performed at the site of treatment or prescribing where there is the opportunity to collect data in a standard of practice care, rather than a controlled environment as with RCTs.

It is not possible to adequately evaluate the safety and effectiveness of a drug without knowing the dose and dosage delivered as treatment. This is true for any method of evaluation. Fortunately, this challenge will be overcome in future evaluations of HBOT by using PlasmaRX and following the principles of Precision HBOT. There will certainly be a shift in the clearance for use process by making O2 dose and dosage data available.

Practice

Impact of Informatics on HBOT

The practice of Hyperbaric Medicine will face many challenges in the rapidly changing Health Care Industry. The primary purpose of the Health Information Exchange (HIE) initiative by The Department of Health and Human Services (DHHS)/ Center for Medicare and Medicaid (CMS) is to ensure EHR systems have the capability to securely share patient information across all providers, and this requires certification by the Office of the National Coordinator (ONC) (51). To be certified, an EHR must store Protected Health Information (i.e. Patient Health Records) that is readily retrieved, fully auditable and secure. All use and sharing must be compliant with HIPAA rules. Another requirement for EHR certification is the capability to aggregate data for different patients to contribute to population health management solutions, predictive analytics, risk management, and care coordination. It is the aggregation of data that is the foundation of the PMI cohort study which has an initial goal of entering 1,000,000 patients (52). Each medical practice must attest to the EHR requirements to be certified by the ONC.

Currently, most medical practice informatics are unable to electronically share patient records across all centers and providers. Due to operating with different software programs, there is frequent incompatibility of the EHR systems between facilities. This lack of interoperability creates poor communication which can result in treatment errors that compromise patient care and safety. It also potentially allows variability in treatment protocols to go unrecognized.

Impact of the transformation of U.S. Health Care on HBOT

Inadequate informatics will cripple providing HBOT services in the Transformation of Clinical Practice Initiative (TCPI) (53) and U.S. Health Care reform in general. PlasmaRx is not only a breathing-gas management system, it is also a robust Electronic Health Records (EHR) system that meets all requirements for Medicare certification as mandated by the Centers for Medicare and Medicaid (CMS) as part of U.S. Health Care reform. It provides documentation containing all the elements required for maximum reimbursement under new merit-based

payment methods that rely on the reporting of quality measures. The PlasmaRx documentation also meets Meaningful Use (MU) objectives and it is fully auditable.

As a Health Information Technology (HIT) system, Plasma Rx is capable of interoperability with different systems used by medical practices. This allows personalized patient information to be shared across all providers involved with the patient's care while using secure and HIPPA compliant end-to-end encryption. It is believed that the coordination of patient care will result in fewer mistakes, less redundancy, cost savings, and improved patient satisfaction. Care coordination is recognized as essential to achieving the Health Care reform Triple Aim goals of better health, better care, and smarter spending.

A recent communication by the UHMS to its members addresses the need for facilities providing HBOT to meet the requirements of the Medicare Access & CHIP Reauthorization Act of 2015 (MACRA). Compliance with MACRA insures eligibility for maximum reimbursements, rather than payment penalties in the future. Payments will be linked with quality measures which are currently being reported through MU and the Physician Quality Reporting System (PQRS). Although this is a direct challenge for providers who are prescribing HBOT, it is likely that payments to facilities delivering treatment will be based on the overall performance of the prescriber and their practice. Fortunately, PlasmaRx provides all the treatment documentation necessary for HBOT facilities to meet this and other challenges in care and payment reform.

At EHI, it is believed that implementation of PlasmaRX and Precision HBOT will make HBOT a model of care delivery in the transformation of the U.S. Health Care System, and the opportunity to begin this initiative is now.

Pharmacovigilance

Pharmacovigilance is defined as "the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other drug-related problem" (54). This regulatory activity is essential to the evaluation of drugs and drug treatments in the "post-marketing" setting and allows for timely recognition and response to potential problems. Evaluation of the safety of a drug relies on the reporting of a potential adverse drug event (ADE)

to the FDA and the drug manufacturer by a physician and other qualified health care provider. It is required that certain ADEs be reported in a timely manner to the FDA by drug manufacturers. The resulting cumulative reported data will then be used to evaluate the safety of a drug and determine if new safety information or warnings must be included by the drug manufacturer in the product information and labeling.

As previously stated, it is not possible to adequately evaluate the safety and effectiveness of a drug without knowing the dose and dosage of the drug to which the patient was exposed. Therefore, PlasmaRx is invaluable to pharmacovigilance in HBOT as the first and only IT system capable of determining the precise dose and dosage of O2 delivered to the patient. The PlasmaRx system can also aggregate data so that potential safety issues will be revealed early and efficacy can be evaluated in an ongoing manner.

Summary

Advancement through science and innovation is needed to overcome certain challenges in HBOT. At EHI, it is believed that now is the ideal time to implement this initiative, given the current supportive regulatory, political and professional environments as well as financial opportunities. It is also excellent timing for alignment with the transformation of the US health care system already underway. At EHI, we are prepared to champion change in Hyperbaric Medicine. It is our mission to advance HBOT through the elucidation of metabolic pathways, development of appropriate protocols, delivery of precision treatment, and compliance with the practice guidelines and goals of health care reform.

We believe the novel PlasmaRx system developed by EHI for delivering O2, monitoring and tracking pressure and %O2, and documenting essential and critical information will revolutionize HBOT and catalyze a new paradigm in Hyperbaric Medicine. Our vision is for PlasmaRx to become the technology platform that facilitates and promotes rapid advancement in HBOT. This platform will be supported by solid scaffolding erected through scientific research and development, and underpinned using the molecular principles of Precision HBOT. It is believed that new standards of care will be realized and established once

PlasmaRx is fully adopted by the HBOT profession. At EHI, we recognize that to accomplish our mission, all stakeholders must be engaged, including patients, clinicians, health care decision-makers, academics, industry, government agencies, policymakers, payers, investors, special interest groups, and others.

Remember: **The answer is right under our nose!**

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