Journal of Controlled Release 209 (2015) 139–149

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Review

Therapeutic gas delivery *via* microbubbles and liposomes

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ARTICLE INFO

Article history:
Received 30 January 2015
Received in revised form 20 April 2015
Accepted 22 April 2015
Available online 23 April 2015

Keywords:
Microbubble
Liposome
Oxygen
Nitric oxide
Xenon
Ultrasound

ABSTRACT

Gaseous molecules including nitric oxide, hydrogen sulfide, carbon monoxide and oxygen mediate numerous cell signaling pathways and have important physiological roles. Several noble gasses have been shown to elicit biological responses. These bioactive gasses hold great therapeutic potential, however, their controlled delivery remains a significant challenge. Recently, researchers have begun using microbubbles and liposomes to encapsulate such gasses for parenteral delivery. The resultant particles are acoustically active, and ultrasound can be used to stimulate and/or image gas release in a targeted region. This review provides a summary of recent advances in therapeutic gas delivery using microbubbles and liposomes.

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http://dx.doi.org/10.1016/j.jconrel.2015.04.027
0168-3659/

1. Introduction

In general, therapeutic gasses have physiochemical characteristics drastically different from those of classic small molecule drugs, offering unique therapeutic advantages and challenges. For instance, these gasses are far smaller than classic drugs and are able to easily diffuse across membranes and through the blood brain barrier. Gasses are rapidly excreted *via* expiration, which reduces toxicity and bioaccumulation concerns compared to classic drugs. A major hurdle, however, is the controlled and site-specific delivery of gasses. The purpose of this review is to provide a comprehensive overview of the use of microbubbles (MBs) and echogenic liposomes (ELIPs) for the delivery of bioactive gasses, particularly oxygen (O_2), nitric oxide (NO), and xenon (Xe).

1.1. Oxygen: therapeutic potential in the reversal of oxygen depletion

It is estimated that at least 50–60% of advanced solid tumors contain hypoxic or anoxic tissue, typically due to irregularities in the tumor microcirculation [1]. Tumor hypoxia is associated with a number of adverse effects, including resistance to chemotherapy and radiation treatment and an increased risk of metastasis. Correspondingly, tumor hypoxia leads to poor prognosis in cancer patients. For example, pancreatic cancer, which is characterized by poorly vascularized tumors, is one of the deadliest human cancers, with a five-year survival rate of less than 6% [2].

Several approaches have been tested in effort to exploit reoxygenation for radiosensitizing hypoxic tumors. Early work involved combining hyperbaric oxygenation with radiation. This approach improved five-year survival rates, but also produced toxicity in healthy tissue [3]. Additional studies have investigated increasing the red blood cell count to increase the O_2 carrying capacity of the blood and therefore increase pO_2 levels in tumors. This approach provided no benefit to head and neck cancer patients [4]. To date, there are no clinically approved methods for increasing tumor oxygen levels for radiosensitzation.

Hypoxemia often presents in cases of severe lung injury, airway obstruction, and acute respiratory distress syndrome, and is associated with increased mortality rates in these patients [5]. Severe hypoxemia is often treated with inspired oxygen, intubation, and mechanical ventilation, however if adequate re-oxygenation is not rapidly achieved, cardiac arrest, organ damage, and death may ensue [6]. In cases of acute blood loss, there is a drastic decrease in systemic oxygen supply and there is a need to restore oxygen delivery to tissues. For this purpose, significant efforts have been made toward developing artificial blood substitutes. These are typically perfluorocarbon emulsions or hemoglobin based oxygen carriers [7,8]. These systems are designed to scavenge oxygen in the high O_2 environment of the lungs and release O_2 content in hypoxic regions, repeating this process as they persist in circulation. A disadvantage of these oxygen delivery platforms is that they require an intact pulmonary function and may not be useful in cases of severe lung injury or airway obstruction.

1.2. Nitric oxide: exploitation of second messenger effects for therapeutic purposes

In 1980, it was discovered that relaxation of vascular smooth muscle cells in response to acetylcholine is dependent on an intact endothelium. Furchgott and Zawadzki defined the molecule responsible as 'endothelium-derived relaxing factor' (EDRF) [9]. Several years later, in the late '80s, it was shown that EDRF is nitric oxide (NO) [10,11]. This discovery sparked intensive research regarding the biological roles of this molecule. It is now known that NO is synthesized endogenously from L-arginine by NO synthases (NOS) of which there are three isoforms: inducible NOS (iNOS), endothelial NOS (eNOS), and neuronal NOS (nNOS) [12].

NO mediates pleiotropic physiological processes through complex and coordinated interactions with multiple cellular targets. It also plays a critical role in the vascular physiology and the cardiovascular system, acting as a vasodilator and inhibiting platelet aggregation [13,14]. Vascular remodeling is mediated by NO, and deficits in NOS/NO pathways may be involved in the development of hypertension and atherosclerosis [15].

NO signaling plays an important role in the central nervous system. It mediates cerebral blood flow, provides neuroprotection, and influences pathophysiological processes post-brain injury [16]. Cerebral NO synthesized in various concentrations and locations elicit diverse and sometimes opposing effects. For example, eNOS-derived NO provides neuroprotection following injury. Whereas, NO derived from iNOS has been shown to exacerbate neuronal injury [16].

The role of NO in cancer biology exemplifies another dichotomy in NO signaling. At low concentrations NO may promote tumor cell growth by stimulating angiogenesis, while at high concentrations NO is cytotoxic and may be a useful chemotherapeutic agent [17].

NO holds therapeutic potential for many conditions including atherosclerosis, hypertension, stroke and cancer. However the concentration and tissue dependence of response is a challenge and presents the risk of side effects. Current approaches to deliver NO include inhalation, intravenous or oral delivery of prodrugs, and the administration of spontaneously releasing chemical donors, among others [18]. There is an extensive body of research surrounding the therapeutic exploitation of endogenous gasses; for a comprehensive review of clinical and preclinical investigations readers are referred to Szabo and Abraham [18].

1.3. Xenon: therapeutic biological effects despite chemical inertness

Xenon, among other noble gasses, illicits significant biological effects. Xenon induces anesthesia through inhibition of *N*-methyl-D-aspartate (NMDA) receptor signaling, and is thought to exert analgesic effects through the same mechanism. Following traumatic brain injury or stroke, over-activation of NMDA receptors triggers biochemical cascades resulting in neuronal death and sustained injury [19]. By inhibiting the NMDA pathway, xenon also provides neuroprotection [20]. Xenon shows promise as a medical gas with potential applications in neuroprotection against stroke or traumatic brain injury and cardioprotection for patients with myocardial infarction. However, adequate delivery is a major hurdle for its clinical translation. The main route of administration currently employed for *in vivo* studies is *via* inhalation. For noticeable neuroprotective effects, Xe must be inhaled at concentrations of 50–70%, which would critically limit the fraction of inspired oxygen and lead to hypoxic tissue damage [21].

2. Microbubbles and liposomes for therapeutic gas delivery

2.1. Protection from endogenous scavengers

The bubble or liposomal shell protects the contained gas from endogenous scavengers. This feature is particularly attractive for the delivery of NO, which rapidly reacts with hemoglobin (reaction rate or $3–5 \times 10^7\ M^{-1}\ s^{-1}$) and consequently has a short half-life in circulation [22]. The particle shell protects NO from scavenging until NO is released (passively or actively *via* ultrasound stimulation). However, once NO is released it must travel to the target site (*i.e.*, endothelium) prior to being consumed by red blood cells (RBCs). There is a RBC-free zone near the endothelium within vessels where NO is able to persist without being consumed by RBCs [22]. According to calculations by Postema et al., targeting NO release in the RBC-free layer may enhance the effectiveness of NO therapy [23]. This may be accomplished by targeting the NO-containing particle to the endothelium using ligands or antibodies or by exploiting acoustic radiation force to push the particles into the RBC-free zone. This will be discussed in more detail later in this review.