Advanced Drug Delivery Reviews 72 (2014) 110–126

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

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

Cardiovascular drug delivery with ultrasound and microbubbles ☆

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ARTICLE INFO

Available online 11 February 2014

Keywords:
Ultrasound
Microbubbles
Fluorocarbons
Perfluoropropane
Perfluorobutane
Perfluoropentane
Cardiovascular
Sonothrombolysis
Drug delivery
Gene delivery
Oxygen delivery

ABSTRACT

Microbubbles lower the threshold for cavitation of ultrasound and have multiple potential therapeutic applications in the cardiovascular system. One of the first therapeutic applications to enter into clinical trials has been microbubble-enhanced sonothrombolysis. Trials were conducted in acute ischemic stroke and clinical trials are currently underway for sonothrombolysis in treatment of acute myocardial infarction. Microbubbles can be targeted to epitopes expressed on endothelial cells and thrombi by incorporating targeting ligands onto the surface of the microbubbles. Targeted microbubbles have applications as molecular imaging contrast agents and also for drug and gene delivery. A number of groups have shown that ultrasound with microbubbles can be used for gene delivery yielding robust gene expression in the target tissue. Work has progressed to primate studies showing delivery of therapeutic genes to generate islet cells in the pancreas to potentially cure diabetes. Microbubbles also hold potential as oxygen therapeutics and have shown promising results as a neuroprotectant in an ischemic stroke model. Regulatory considerations impact the successful clinical development of therapeutic applications of microbubbles with ultrasound. This paper briefly reviews the field and suggests avenues for further development.

Contents

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Ultrasound triggered drug delivery".

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http://dx.doi.org/10.1016/j.addr.2014.01.012

1. Introduction

Microbubbles are FDA approved in the US with indications for echocardiography [1]. Work is underway to gain approval in the US for radiology indications as well, but at the time of preparation of this review, microbubbles are not yet approved for radiology ultrasound imaging in the US [2]. In Europe and Canada, however, microbubbles are approved for both echocardiography and radiology indications [3,4]. Microbubbles present an acoustic impedance mismatch between biological fluids and tissues, are highly compressible and are highly reflective to ultrasound; hence microbubbles are highly effective as contrast agents for diagnostic ultrasound [5–7]. The purpose of this review is to present the potential therapeutic applications of microbubbles within the cardiovascular field and the diagnostic applications of approved microbubble-based contrast agents will not be covered by this review. As targeted microbubble agents might also be used for therapeutic as well as diagnostic molecular imaging applications, however, this review will also cover targeted microbubbles for cardiovascular applications [8,9]. In addition to their role as therapeutic agents with ultrasound, microbubbles can also be used for oxygen delivery and this review will present some of these potential applications for treating cardiovascular disease as well [10,11].

The microbubbles that are currently approved or in clinical trials all contain fluorinated gases. Coatings of lipid, protein or polymer shell stabilize them. There are currently two FDA approved microbubble-based agents in the US, Definity® and Optison®. A layer of phospholipid coats Definity microbubbles and Optison microbubbles are coated by a layer of denatured human serum albumin. The coating material envelops the microbubbles and helps to control microbubble size as well as to maintain microbubble stability. Another agent, EchoGen®, was composed of dodecafluoropentane emulsion (DDFPe) nanodroplets stabilized by a fluorosurfactant, PEG-Telomer-B [12,13]. The boiling point of DDFP is about 28 °C. Because of surface tension effects, DDFPe did not truly form microbubbles following IV injection, and had to be activated (e.g. hypobaric activation by pulling back on the syringe to create negative pressure and thereby create microbubbles for use as an ultrasound contrast agent [14]). EchoGen was approved by the European Medicines Agency (EMEA) and approvable by the US FDA but never launched [15]. The currently approved agents in the US are based upon perfluoropropane (boiling point about −34 °C). Sonazoid® which is approved in Japan is based upon perfluorobutane (boiling point about −1.4 °C) and BR-14 and BR-55 are also based upon perfluorobutane [16–18]. Microbubbles either approved by the FDA, the European Medicines Agency, or currently in clinical trials are described in Table 1.

Fluorinated gases are used in the above agents because of the low solubility of these materials in aqueous media. The fluorinated gases are less soluble than air, nitrogen or oxygen. The less soluble gases dissolve more slowly affording production of longer-lived microbubbles useful for ultrasound imaging and also potentially for cardiovascular drug delivery [25]. In general, the higher the molecular weight of the gas, the lower the solubility. Of the gases shown in Table 1 perfluoropentane is the least soluble, perfluorobutane the next least soluble, sulfur hexafluoride the most soluble and perfluoropropane intermediate. As shown in Table 1, two of the approved microbubble products have phospholipid coatings and one of the microbubble products is stabilized by denatured albumin protein. One of the products, Sonozoid, is coated with phosphatidylserine (PS), an anionic form of phospholipid [21]. PS is accumulated by macrophages and this agent is used for liver imaging [26]. Two products are in clinical trials in Europe, both based upon phospholipid-coated perfluorobutane microbubbles [17,18]. BR-55 also contains a lipopeptide targeted to the receptor for vascular growth factor (VEGFR2) [18].

The mean size of Definity (perflutren) microbubbles is around 1 μm but particles range in size from several microns to submicron. Definity is prepared by agitation of a sealed vial of phospholipids and a headspace of perfluoropropane gas. The mean size of the microbubbles in one study was about 3–4 μm immediately after agitation and preparation and about 2 μm more than 24 h later [27]. In another study of Definity, the mean size of the microbubbles changed over a period of 3 h from about 3 μm to 0.98 μm with increasing decanting time [28]. The mean size of Optison is probably larger, with a mean size listed on the prescribing information of 3–4 μm and 95% of particles <10 μm and few particles as large as 32 μm [20]. The measured size of the microbubbles depends in part on the measurement system. Some systems such as quasi-elastic light scattering are more sensitive to sub-micron sized particles while other systems, e.g. optical particle sizing and light-obscuration systems, are more sensitive to particles larger than a micron. We show particle sizing for DDFPe (NuvOx Pharma, Tucson, AZ), identical to EchoGen described above, except that DDFPe contains a buffer helping to stabilize the formulation (EchoGen was unbuffered) (Fig. 1) [10]. Sizing of DDFPe with dynamic light scattering reveals a mean particle size of about 296 nm. Particles larger than 1 μm are essentially invisible to the dynamic light scattering system [29]. The light obscuration system (e.g. Accusizer) shown below, however, is sensitive to particles ranging from about 0.5 μm up to about 500 μm in size. For most microbubble preparations, which predominantly contain particles over 1 μm in size, the light obscuration kind of system is probably most appropriate. To characterize a formulation that contains a substantial population of submicron particles, e.g. Definity or EchoGen, both kinds of particle sizing systems are necessary to fully characterize the microbubble preparation [29,30]. Note that the largest particles, e.g. >10 μm in size are most apt to cause adverse bioeffects, and particle sizing is therefore an important measure to ensure product safety [31,32]. We have used the Accusizer to study DDFPe after hypobaric activation and mean particle size increases to about 2.2 μm (unpublished data).

1.1. Cavitation

Depending upon the acoustic intensity of the ultrasound used to insonate the microbubbles, the microbubbles may oscillate. The

Table 1
Microbubble contrast agents that are approved or currently in clinical trials.

Agent	Company	Coating	Gas	Place approved
Definity [19]	Lantheus	Phospholipid	Perfluoropropane	US and Canada
Optison [20]	GE Healthcare	Human serum albumin	Perfluoropropane	US
Sonazoid [21]	GE Healthcare	Phosphatidylserine	Perfluorobutane	Japan
Sonovue [22]	BRACCO	Phospholipid	Sulfur hexafluoride	Europe
BR-14 [17]	BRACCO	Phospholipid	Perfluorobutane	Clinical trials in Europe
BR-55 [18]	BRACCO	Phospholipid/lipo-peptide	Perfluorobutane	Clinical trials in Europe
EchoGen [12]	Sonus	PEG-Telomer-B	Dodecafluoropentane	EMEA*
Imagify [23]	Acusphere	Poly-lactic glycolic acid [24]	Perfluorobutane	MAA to EMEA**

* EchoGen was approved by the EMEA and approvable by the FDA. The corporate sponsor voluntarily withdrew the product from the EMEA. A reformulated version of DDFPe is currently under development by NuvOx Pharma, Tucson, AZ, as an oxygen therapeutic [10].
** The Market Authorization Application (MAA) was recently submitted by Acusphere to the EMEA for Imagify [24].