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Sonodynamic antimicrobial chemotherapy: First steps towards a sound approach for microbe inactivation

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ABSTRACT

Sonodynamic therapy (SDT) relies on the ability of ultrasound to activate sonosensitisers and trigger the generation of reactive oxygen species (ROS) to achieve cell death. SDT was explored as an anticancer approach until 6 years ago, when its potential application as an antimicrobial strategy was pointed out and the term "sonoantimicrobial chemotherapy" (SACT) was coined. The excellent penetration of ultrasound in liquid media make SACT a particularly promising approach for the non-invasive treatment of deep-seated infections, and for the reduction of bacterial load in turbid water. In this review we provide an account of the brief history of SACT, from its molecular bases to the current state of the art and perspective applications.

1. Introduction

Over the last few decades the insurgence of antibiotic-resistant microorganisms has become a pressing concern for public health as it represents the main cause for the failure of antibiotic therapy [1]. The scientific community devoted great efforts to provide alternative solutions not only to eradicate infections in the medical and veterinary setting, but also to reduce bacterial loads in the context of environmental and industrial applications, since agricultural/environmental use of antibiotics plays a pivotal role in the spreading of resistance, through food-borne microorganisms [2] and wastewater [3–5]. In the quest for alternative approaches to microbial inactivation, physical methods (*e.g.*, irradiation, heat, high pressure, etc.) gained popularity because they typically have less potential to induce resistance, and are amenable to application on large scale [6–8]. Amongst these methods, ultrasonic waves have been explored as a bactericidal tool since Harvey and Loomis provided the first evidence of the lethal effect of ultrasound on microbes in 1929 [9]load in turbid water. In this review we provide an account of the brief history of SACT, from its molecular bases to the current state of the art and perspective applications Following their work, studies on ultrasound-mediated bacterial inactivation using a plethora of different experimental conditions (*e.g.*, continued/pulsed waves, low/high intensity wave, different types of ultrasound generators, etc.) were published, often reporting conflicting results. Nevertheless, some general traits emerged, such as the higher susceptibility of Gram-positive *vs.* Gram-negative bacteria, of rod-shaped bacteria *vs.* cocci, and of larger *vs.* smaller bacteria [10]. It was shown that ultrasound interferes with the life cycle of both planktonic [11,12] and sessile (biofilm) [13] bacteria, but again, depending on the microbe and the irradiation conditions, the outcome can be that of cell replication inhibition or cell inactivation, but also of the stimulation of microbial vitality [14].

Ultrasound-mediated microbial inactivation eventually made its way into applications in medicine and industrial processes. Sonication, both stand-alone and associated with heath or high pressure, has been successfully employed to reduce the bacterial load in foodstuff with no detrimental effect on the quality of the treated food [15–17]. In the medical field, ultrasound-based scalers are employed in dentistry for plaque removal [18], to promote the microbial uptake and/or the release of antibiotic (*e.g.*, vancomycin, gentamicin) from prosthetic implants [19,20].

Until a few years ago, the paths of antimicrobial photodynamic therapy and ultrasound waves hardly crossed. The synergy of ultrasound and PACT was explored as a way to enhance the efficacy of photodynamic treatment in the disinfection of infected wounds

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[21,22], and in a combined photodynamic/ultrasound treatment to eliminate dental plaque [23]. High-frequency ultrasound was also employed to assess the efficacy of PACT in vitro [24]. While these reports were presenting light and ultrasound as the physical triggers of different biological effects (*i.e.*, microbe inactivation and enhanced cell uptake or imaging, respectively), evidence coming from the field of anticancer therapy showed that in the presence of photosensitisers, ultrasound and light could trigger similar events and elicit similar biological responses [25]. Such evidence formed the basis of a new anticancer approach named of sonodynamic therapy (SDT), which few years ago crossed borders to enter the field of antimicrobial therapy. The aim of this review is to give an account of the state of the art of antimicrobial SDT, and an overview of the molecular bases of its mechanism of action.

2. Ultrasound in living systems

Ultrasound is an acoustic radiation inaudible to humans, with a frequency exceeding 20 kHz. Ultrasound generates a mechanical vibration characterised by repeated cycles of compression and expansion in the surrounding environment [27]. The ability of ultrasound to propagate through tissue and induce transient or permanent changes to biomolecules and cells has been successfully exploited for numerous biomedical applications, including therapeutic intervention (tissue repair, thrombolysis, angioplasty, drug delivery, endodontic disinfection, etc.) and diagnostic techniques [26] (Fig. 1). Modern ultrasound generators for biomedical applications rely on piezoelectric devices to produce a focused ultrasound beam that can be delivered to the target environment through the skin and underlying tissues. The ultrasound frequency range employed for diagnostic purposes is rather broad (2.0–28.0 MHz), whereas therapeutic applications are typically carried out with waves within the 0.5–3.0 MHz frequency range [27]. Ultrasound-based diagnostic techniques rely on low-energy irradiation to avoid damaging cells/tissues, whereas therapeutic intervention requires the delivery of higher doses of energy to generate the desired biological outcome [28].

The interactions of ultrasound with living systems occur *via* three main pathways, namely, *thermal* (*i.e.*, heat generation), *chemical* (*i.e.*, radical formation) and *mechanical* (*i.e.*, shear stress, liquid jets, shock wave), each of which triggers specific effects. The *thermal* effects are the desired outcome of most ultrasound-based therapeutic applications, as it happens, for example, in the high-intensity focused ultrasound (HIFU) therapy of cancer, where an ultrasound beam focused on the target malignancy delivers a dose of energy that causes a spatially-confined hyperthermic effect and subsequent coagulative necrosis at the focal point [29]. The *mechanical* effects of ultrasound causes transient alterations of the permeability of cell membrane, a phenomenon that underpins the enhanced cell uptake of both low- and high-molecular weight drugs observed upon exposure to ultrasound [30]. The phospholipid membrane is intrinsically able of absorbing the mechanical energy generated by the sonic wave, and of responding with deformations (compressions and expansions) of the intramembrane space: this behaviour has been advantageously exploited in the ultrasound-mediated drug targeting and controlled drug release in several therapeutic fields [31]. The *chemical* effects of ultrasound are associated to the onset of sonochemical reactions (*e.g.*, ionisation, electron-transfer), and/or the sonolytic formation of free radicals: these processes have a great potential for therapeutic applications, but due to the short lifetimes of the species generated, they are very challenging to harness.

It is generally agreed that the effects of ultrasound in tissues originate from a phenomenon known as acoustic cavitation. The action of ultrasound waves propagating in a liquid promotes the formation of gas- or vapour-filled cavities (microbubbles), which undergo shrinking and growing cycles due to the alternate compression and expansion generated by the different pressure phases of the ultrasound wave. Low-amplitude oscillation of the microbubbles leads to stable cavitation (non-inertial), in which the microbubble "pulsates" generating microstreams in the surrounding medium, causing shear stress to cell membranes and eventually resulting in the transient formation of pores (sonoporation) (Fig. 2A). High-amplitude oscillations of the microbubble give rise to inertial cavitation, in which the alternating shrinking and expansion increase in intensity until the microbubble implodes. Collapsing microbubbles results in the generation of shock waves and/or liquid jet formation, the force of can easily perforate cell membranes. (Fig. 2B and C).

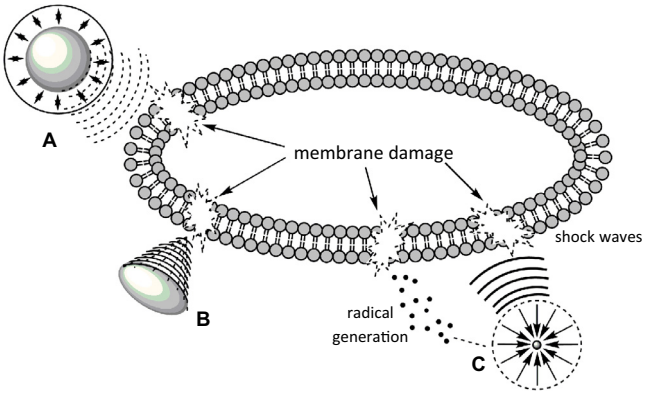

Fig. 2. Membrane damages following acoustic cavitation.

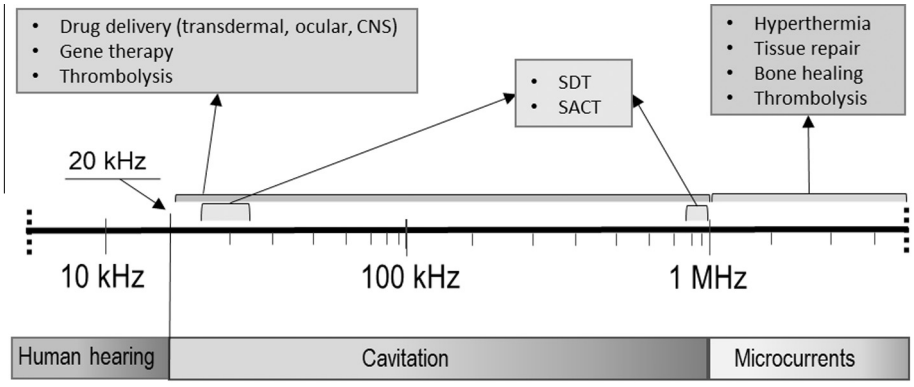

Fig. 1. Simplified diagram of the ultrasound frequencies used for therapeutic applications Ref. [26].