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Sounding the death knell for microbes?

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Over the past 5 years, several studies showed that ultrasound, which is sound with a frequency >20 kHz, is able to kill bacteria by activating molecules termed sonosensitizers (SS) to produce reactive oxygen species, which are toxic to microbes. It is our opinion that this work opens up the potential for the development of a novel form of ultrasound-mediated antimicrobial therapy. Termed sonodynamic antimicrobial chemotherapy (SACT), we define this therapy as a regime where a SS is selectively delivered to target microbial cells and activated by ultrasound to induce the death of those microbial cells. Here, we review recent work on SACT, current understanding of its mechanisms, and future prospects for SACT as a therapeutically viable antimicrobial regime.

The origins of sonodynamic antimicrobial chemotherapy

Photosensitizers (PS) are molecules that are activated by light and can be preferentially taken up by cancer and microbial cells [1–4]. Based on these properties, PS have become well-established therapeutic agents that are used in photodynamic therapy to kill cancer cells [5,6] and in photodynamic antimicrobial chemotherapy (PACT) to eradicate microorganisms [1,2]. Ultrasound is inaudible to humans and is defined as sound with a frequency >20 kHz. In around 1990, it was found that ultrasound was able to activate PS to kill cancer cells [7–9]. This ability was subsequently developed into an anticancer modality known as sonodynamic therapy (SDT), with PS being more generally referred to as sonosensitizers (SS) [10–12]. However, in 2009 it was predicted that if the combination of ultrasound and SS was able to kill cancer cells, then it may also be able to eradicate microbial cells, as seen with the activation of PS by light [13]. A few years later, this prediction was confirmed by several experimental studies [14,15], which, in our opinion, opens up the potential for the development of a novel form of ultrasound-mediated antimicrobial activity, termed sonodynamic antimicrobial chemotherapy (SACT) [13]. By analogy to PACT, we define SACT as a regime where a SS is selectively delivered to target microbial cells and activated by ultrasound to induce the death of those cells. However, the ethos encapsulated in this definition is a future goal for which SACT is striving, and currently it is an embryonic modality. Here, we review progress towards this goal with reference to recent work on SACT, current understanding of the mechanisms underpinning the modality, and future prospects for SACT as a therapeutically viable antimicrobial regime.

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SACT and antimicrobial activity

Human interest in ultrasound has a long and international history, which goes back to the 1770s, and has led to its exploitation for a variety of medical purposes which currently range from bone cutting to promoting drug delivery (Table 1). However, the antimicrobial application of ultrasound is generally taken to have begun in 1927, when it was demonstrated that ultrasound could destroy the algal microorganism *Spirogyra* [16]. Since these earlier studies, ultrasound, both alone and in combination with other antimicrobial agents and strategies, was found to be able to eradicate a variety of other microorganisms, ranging from bacteria to viruses. Based on this ability, ultrasound is used in a diverse spectrum of antimicrobial strategies, ranging from decontamination in the food industry to antibiotic therapy in relation to indwelling medical devices (Table 1) [17]. The therapeutic potential for SACT emerged in the early years of the present decade, when ultrasound was shown to be able to induce antibacterial activity by a variety of molecules [14,15].

In 2011, the first of these studies investigated the susceptibility of *Escherichia coli* to Ciprofloxacin and Levofloxacin [14], which are better known as conventional antibiotics but have been shown to also function as SS [14,18]. In the absence of ultrasound, it was found that both molecules were able to induce considerable reductions in the viability of *E. coli*, which were of the order of 75%, as might be expected from the antibiotic capacity of these molecules. In contrast to this, the application of ultrasound alone had only a minor effect on the viability of the organism. However, when ultrasound was applied simultaneously with Ciprofloxacin and Levofloxacin, a strong synergistic antimicrobial effect was observed, which under some experimental conditions resulted in the eradication of these organisms at levels approaching 95% [14]. Similar results were obtained in the second of these studies, which in 2013 investigated the ability of an established SS, Rose Bengal (RB), to function as a SACT agent. This ability was confirmed when it was found that the separate application of RB and ultrasound led to minor reductions in the viability of both *E. coli* and *Staphylococcus aureus*, but

Table 1. Major uses of ultrasound (adapted from [17])

Applications of ultrasound		Refs
Antimicrobial applications of ultrasound		
High power	Sonodynamic disinfection	[44]
	Endodontic irrigation	[45]
Low power	Sonodynamic antibiotic therapy	[46]
	Sonocatalytic disinfection	[47]
	Sonodynamic antimicrobial chemotherapy (SACT)	[17]
Therapeutic applications of ultrasound		
High or intermediate power	Dentistry and surgery (scalpel and bone cutting)	[48]
	Synthesis of microcapsules for drug delivery	[49]
Intermediate or low power	Separation technology	[50]
	Physiotherapy	[51]
	Destruction of blood clots	[52]
	Transdermal drug delivery (sonophoresis)	[53]
	Sonodynamic therapy (SDT)	[12]
	High intensity focused ultrasound	[54]
	Improved uptake of molecules (sonoporation)	[55]
	Orthodontics	[56]
Low power	Diagnostic imaging	[57]

when applied in combination a strong synergistic eradication of both bacteria was observed, which under some experimental conditions occurred at levels approaching 100% [15].

SACT: mechanisms

Currently, the mechanisms by which activated SS kill microbes are poorly understood [14,15]. Insight into these mechanisms gained from SDT studies have established that the activation of SS involves acoustic cavitation [10–12], an adiabatic process that involves the collapse of gas bubbles induced by ultrasound in the liquid medium [19,20]. It has been estimated that, on a nanosecond time scale, collapse cavitation can induce upwards of temperatures of approximately 5000 K and pressures of 250 MPa [21], extreme conditions that can generate high liquid shear force, shock waves, localized heating, and free radicals [19,20]. All of these phenomena appear to be able to contribute to the anticancer activity of SS via several mechanisms, including sonoporation, sonochemistry, and sonoluminescence [10–12].

Sonoporation essentially involves the ultrasound-mediated perforation of membranes [22] and earlier studies on SDT proposed that the shearing effect of bubble pulsations on cancer cell membranes synergizes the ability of SS to permeate and destabilize these membranes, resulting in cancer cell death [23,24]. However, many studies have proposed that the damage inflicted in SDT involves the activation of SS to induce the production of reactive oxygen species (ROS) [10–12], which are forms of oxygen that can react with electron-rich cellular components, such as membrane lipids, DNA, and proteins [25]. The involvement of ROS in SDT is well established and several mechanisms have been proposed to explain their generation and cellular sites of action [10–12]. One early study proposed that the anticancer activity of SDT resulted from the activation of SS via sonochemical reactions in which SS-derived ROS are produced either by direct pyrolysis or via reactions with other ROS formed by the pyrolysis of water. These SS-derived ROS then react with dissolved oxygen to form other ROS, including peroxyl and alkoxyl radicals, which are able to initiate damage to critical cellular sites (Figure 1) [26].

However, the most commonly proposed mechanism for the anticancer activity of SDT is the production of ROS through the direct electronic excitation of SS by sonoluminescence [10–12], which are flashes of light that

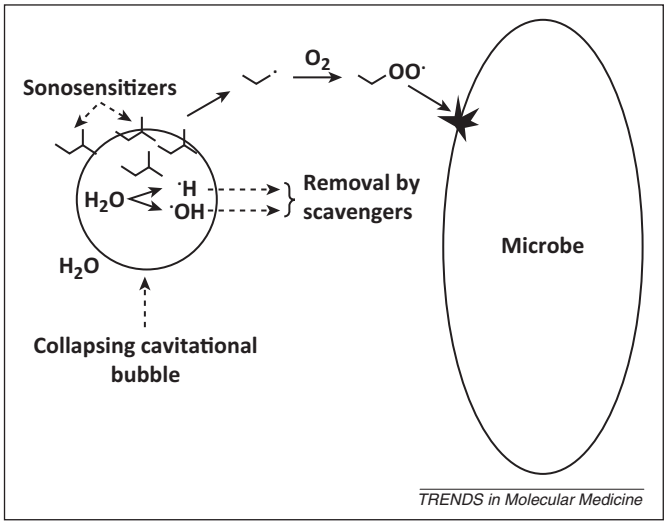

Figure 1. The activation of sonosensitizers (SS) by sonochemistry. Adapted from [26] and represents the activation of SS by sonochemistry in sonodynamic antimicrobial chemotherapy (SACT). In this representation, a SS undergoes pyrolysis inside collapsing cavitation bubbles or in the heated gas–liquid interface, forming free radical intermediates. These intermediates, which are predominantly carbon-centered, react with dissolved O_2 to form peroxyl radicals, capable of attacking critical cellular sites in microbial cells owing to their ability to diffuse significant distances. In contrast to this, free radicals, which are also formed during cavitation collapse through the sonochemistry of water, are unable to cause significant cellular damage to microbes owing to their extremely high reactivity and hence short diffusion distances.