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Review paper

Sonodynamic therapy for the treatment of atherosclerosis

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ABSTRACT

Atherosclerosis (AS) is a chronic inflammatory disease of large and medium-sized arteries that leads to ischemic heart disease, stroke, and peripheral vascular disease. Despite the current treatments, mortality and disability still remain high. Sonodynamic therapy (SDT), a non-invasive and localized methodology, has been developed as a promising new treatment for inhibiting atherosclerotic progression and stabilizing plaques. Promising progress has been made through cell and animal assays, as well as clinical trials. For example, the effect of SDT on apoptosis and autophagy of cells in AS, especially macrophages, and the concept of non-lethal SDT has also been proposed. In this review, we summarize the ultrasonic parameters and known sonosensitizers utilized in SDT for AS; we elaborate on SDT's therapeutic effects and mechanisms in terms of macrophages, T lymphocytes, neovascularization, smooth muscle cells, lipid, extracellular matrix and efferocytosis within plaques; additionally, we discuss the safety of SDT. A comprehensive summary of the confirmed effects of SDT on AS is conducted to establish a framework for future researchers.

1. Introduction

Atherosclerosis (AS) is the most common underlying pathogenesis of cardiovascular disease, cerebrovascular disease, and peripheral artery disease (PAD) [1]. Myocardial infarction and stroke, as the most common complications of these diseases, represent the leading cause of death worldwide [1,2]. Currently, the treatment of carotid atherosclerotic plaque primarily involves pharmacological and surgical interventions. Lipid-lowering drugs, known as statins, are extensively utilized; however, their duration is prolonged and the most severe systemic adverse effect observed is myotoxicity [3]. Surgical treatment includes carotid artery stenting and carotid endarterectomy, but both are invasive and reserved for patients with moderate to severe carotid artery stenosis who meet specific indications [4]. Sonodynamic therapy (SDT) is a treatment that uses ultrasound to locally stimulate sonosensitizers to exert biological effects, which has the advantages of non-invasive and no systemic side effects. SDT has been developed as a promising alternative treatment for inhibiting the progression of atherosclerotic plaque [5]. One of the major focuses of recent researches in this field has been to explore the mechanism, laying the foundations for further clinical applications. However, the mechanisms are related to sonosensitizer species and ultrasound parameters [6]. This review focuses on sonosensitizer species, ultrasound parameters, the underlying function, mechanism and safety of SDT in treating AS.

2. Sonodynamic therapy

SDT is a non-invasive treatment using low-intensity ultrasound to locally activate sonosensitizer to produce reactive oxygen species (ROS), which alters cellular fate or function. Developed from the photodynamic therapy (PDT), SDT has greater penetrability and more focused effects on the lesion [7]. Consequently, SDT has emerged as a prominent area of research.

2.1. Ultrasound irradiation

Non-invasive, low-intensity ultrasound has been widely used in the therapeutic research for various diseases. Ultrasonic parameters vary among different diseases. The approximate parameter range of the ultrasound system in SDT on AS is as follows:

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frequency, 1.0–1.5 MHz; intensity, 0.1–2.1 w/cm^2; repetition frequency, 100 Hz; duty cycle, 5%–10%; exposure time, 2–15 min. The intensities of SDT used in patients were the highest, followed by animals and then cells with the lowest intensity. This may be related to attenuation of ultrasound intensity as it passes through tissue. The effective ultrasonic parameters for SDT treatment are a tricky problem affected by the type of sonosensitizers and biological model (the absorption rates of sonosensitizers are different vary across different biological models). Therefore, under the unified conditions, numerous experimental analyses are required to determine the parameters.

Moreover, traditional single-element therapeutic transducer has a single acoustic field, making it difficult to position therapeutic and imaging transducers in the same location during ultrasound imaging-guided SDT. Recently, Wang et al. [8] designed and fabricated a "concentric ultrasound transducer for theranostics" (CUST-T), which includes a central 8-MHz linear array transducer for ultrasound imaging, and a peripheral 1-MHz hollow two-dimensional planar array transducer for generating phased-array focused ultrasound. The study demonstrated that CUST-T provided sufficient image guidance for accurate SDT of atherosclerotic plaques in peripheral arteries, making it applicable in clinical practice.

2.2. Sonosensitizers

Sonosensitizer should possess certain features, including sensitivity to ultrasound, specific aggregation at the lesion, and low toxicity to healthy tissue. It is very interesting that most photosensitizers used in PDT can also be utilized as sonosensitizers for SDT. While there are many sonosensitizers used for tumors, few sonosensitizers related to AS have been reported yet. Sonosensitizers utilized in AS SDT so far include protoporphyrin IX (PpIX) [9–11], 5-aminolevulinic acid (5-ALA) [5,12–18], sinoporphyrin sodium (DVDMS) [19–21], berberine (BBR) [22], hypericin (HY) [23,24], hydroxysafflor yellow A (HSYA) [25,26], curcumin (Cur) [27], hydroxyl acylated curcumin (HAC) [28], curcumin nanosuspensions (Cur-ns) [29], cyclodextrin polymer (CDP) [30], methyl ester of aminolevulinic acid gold nanoparticles (MALA: AuNPs) [31], PFP-HMME@PLGA/$MnFe_2O_4$-ramucirumab nanoparticles (PHPMR NPs) [32] and hyaluronic acid (HA)- and polyethylene glycol (PEG)-modified CuS/TiO_2 heterostructure nanosheets (HA-HNSs) [33] (Table 1) [5,9–33].

Taken together, although the number of sonosensitizers applied in AS treatment is far less than those used in tumors, they are constantly evolving. ALA and DVDMS have already been tested in small-scale pilot clinical trials to inhibit AS and have proven to be safe and effective as sonosensitizers. Nanotechnology has rapidly developed due to its advantages of stabilization, micro, target and high efficiency in researches. However, further researches are needed for the clinical translation of these sonosensitizers.

3. The fundamental mechanism of SDT

The main mechanism of SDT is oxygen radical theory. In 1989, Yumita et al. [34] discovered that the key mechanism by which SDT kills target cells is to generate ROS. Studies have verified that SDT induces intracellular generation of ROS and further produces a series of effects in atherosclerotic plaques [5,11,13,18,22,24,29,31,35]. It is now clear that SDT could induce the electron transition

Table 1
Sonosensitizers used in sonodynamic therapy (SDT) for the treatment of atherosclerosis.

Sonosensitizer	Category/Source/Composition	Characteristics	Research subjects	Refs.
PpIX	Haematoporphyrin derivative	Selective accumulation in atherosclerotic plaque, and 10 times higher than in normal vessel walls	Macrophages, vascular smooth muscle cells	[9–11]
5-ALA	PpIX's natural precursor	Uptake by macrophages; metabolism into PpIX in mitochondria; ALA-PpIX selective accumulation in atherosclerotic plaque, and 10–12 times higher than in normal vessel walls	Macrophages, mice, rabbits, patients	[5,12–18]
DVDMS	Porphyrin derivative, isolated from Photofrin	Accumulation in macrophages of plaque; stronger sonoactivity than that of several other porphyrins; generating reactive oxygen species more efficiency than PpIX	Macrophages, mice, rabbits, patients	[19–21]
BBR	A molecule isolated from Chinese herbal medicine Coptischinensis	Uptake by macrophages	Macrophages	[22]
HY	Extract of Chinese herbal medicine St John's worts	Uptake by macrophages	Macrophages	[23,24]
HSYA	Extract of traditional Chinese herbal medicine safflower plants	Uptake by macrophages; higher water solubility and safety compared to HY, Cur, and HAC	Macrophages	[25,26]
Cur	Extract of traditional Chinese herbal medicine Curcuma longa	Low bioavailability and instability	Macrophages	[27]
HAC	Acetylation of the hydroxyl of Cur	More stable than Cur	Macrophages	[28]
Cur-ns	Nanosonosensitizer; composed of Cur, polyvinylpyrrolidone and sodium dodecyl sulfate	Improving the insolubility and low bioavailability of Cur	Macrophages, mice	[29]
CDP	Nanosonosensitizer; a polymeric formulation of cyclodextrin	Accumulation within plaques; improving pharmacokinetics and plaque targeting of cyclodextrin	Macrophages	[30]
MALA: AuNPs	Nanosonosensitizer	AuNPs as carriers for MALA; MALA improving cellular uptake and elevating intracellular PpIX production compared to ALA	Macrophages	[31]
PHPMR NPs	Nanosonosensitizer; a nanoplatform for multimodality imaging-guided SDT	Actively targeting the mitochondria of endothelial cells and increasing nanoparticle accumulation in plaque neovessels	Endothelial cells, rabbits	[32]
HA-HNSs	Nanosonosensitizer; a sonodynamic and photothermal agent	Targeting intraplaque macrophages; utilized for sonodynamic/photothermal synergistic therapy	Macrophages, mice	[33]

PpIX: protoporphyrin IX; 5-ALA: 5-aminolevulinic acid; DVDMS: sinoporphyrin sodium; BBR: berberine; HY: hypericin; HSYA: hydroxysafflor yellow A; Cur: curcumin; HAC: hydroxyl acylated curcumin; Cur-ns: curcumin nanosuspensions; CDP: cyclodextrin polymer; MALA: AuNPs: Methyl ester of aminolevulinic acid gold nanoparticles; PHPMR NPs: PFP-HMME@PLGA/$MnFe_2O_4$-ramucirumab nanoparticles; HA-HNSs: hyaluronic acid (HA)- and PEGpolyethylene glycol (PEG)-modified CuS/TiO_2 heterostructure nanosheets.

by irradiating the target tissue with ultrasound and energizing the sonosensitizer retention inside. When the transitioned electron returns to ground state, there appears the ROS [7]. Currently, there are two hypotheses regarding the mechanism for generating ROS as a result of stimulating sonosensitizers.

3.1. Sonoluminescence hypothesis

Sonoluminescence occurs when internally generated and enlarged bubbles implode in a liquid under the excitation of ultrasonic waves, emitting bursts of light that stimulates sonosensitizers to produce ROS. This process of bubble expansion and collapse is known as cavitation effects, which include stable and inertial cavitation. Stable cavitation is the generation of mild bubbles, mostly under continuous vibratory and oscillatory states. Inertial cavitation refers to a relatively strong bubble dynamic process. Inertial cavitation bubbles undergo a process of rapid contraction to collapse, absorbing a large amount of acoustic energy, and then energy was released in a small area. This results in localized high temperature and pressure, a strong shock wave, high-speed micro-jets and formed free radicals in the liquid. Inertial cavitation is more closely associated with the generation of ROS. The emission of light during the process of cavitation effects is likely related to blackbody radiation, bremsstrahlung, recombination radiation or complex radiation. This hypothesis was proposed by Pickworth, but the accurate mechanism remains unclear [36].

3.2. Pyrolysis hypothesis

The rupture of bubbles gives rise to a brief heat of 10,000 K, which breaks down sonosensitizers and generates free radicals. These free radicals further react with some endogenous substances to produce ROS. As a result, the sonosensitizer gets excited and produces ROS.

It has also been found that the 18 kDa mitochondrial translocator protein (TSPO) participates in the generation of ROS. TSPO, located in the outer mitochondrial membrane, is associated with adenine nucleotide translocator and voltage dependent anion channel, which are the core components of the mitochondrial permeability transition pore (mPTP). Additionally, TSPO has a high-affinity recognition site for porphyrins, particularly PpIX [17]. TSPO is associated with the transport of porphyrins, such as PpIX across through the mitochondrial membrane. It has been found that ultrasound activation of ALA-PpIX binding to TSPO produces ROS [17] (Fig. 1). SDT finally causes a large accumulation of ROS that cannot be timely removed and are retained in cells, resulting in a series of effects.

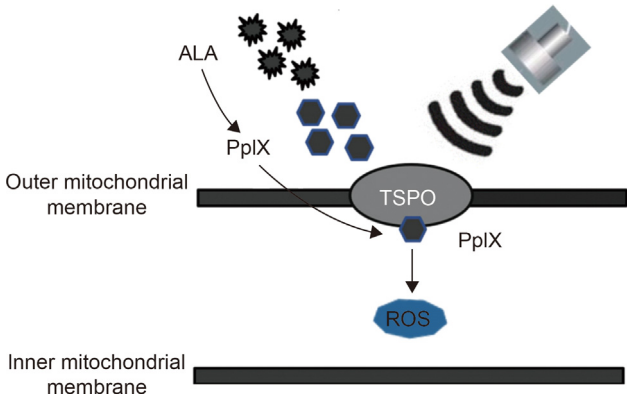

Fig. 1. The main mechanism of sonodynamic therapy (SDT) in generating reactive oxygen species (ROS). Ultrasound activates the binding of 5-aminolevulinic acid derived protoporphyrin IX (ALA-PpIX) to the 18 kDa mitochondrial translocator protein (TSPO), resulting in the generation of ROS.

4. SDT induces the inhibition of atherosclerotic plaque progression

Several studies have demonstrated that SDT inhibits the progression of atherosclerotic plaque. It has been proven that SDT can decrease the percentage of lumen stenosis and alter the composition of plaque to stabilize plaques including reducing the necrotic core area and so on [5,13,15,16,32,37]. It is generally recognized that SDT is a local treatment without systematic response. Sun et al. [13] also showed in a pilot clinical trial that ALA-SDT does not change serum cholesterol levels. However, one study proved that Cur-ns-SDT significantly reduced the level of low-density lipoprotein and total cholesterol level in apoE$^{-/-}$ mouse [29]. The potential reason could be attributed to the relatively small size of the mouse and the extensive coverage area of the ultrasound probe during treatment. Currently, SDT has been applied in phase I/II clinical trials for the treatment of patients with carotid AS (NCT03871725, and NCT03382249) and PAD patients with intermittent claudication (NCT03318484, and NCT03457662). In 2019, it is reported that the percentage of diameter stenosis decreased in PAD patients following combination therapy of atorvastatin and ALA-SDT for 4 weeks, and continued to decrease until 40 weeks. However, the group treated with atorvastatin alone did not show any significant changes [13]. In 2020, it was reported that combined treatment of DVDMS-SDT and atorvastatin is more effective than atorvastatin alone in PAD patients, as evaluated by arterial inflammation analysis using positron emission tomography/computed tomography (PET/CT) and walking functional performance examination [19]. Jiang et al. [19] confirmed that DVDMS-SDT can rapidly reduce plaque inflammation and improve functional performance in patients with lower limb PAD. The greater the inflammatory response of plaque, the better the efficacy. Additionally, SDT also had good long-term efficacy. Yao et al. [21] also conducted a small-scale pilot study on patients with PAD, and the results were evaluated using contrast-enhanced ultrasonography and PET/CT. After one month of SDT treatment, a significant reduction in neovascularization and arterial inflammation was observed, which was almost equivalent to the therapeutic outcome of 3-month intensive statin treatment (Table 2) [13,19,21]. The findings suggest that SDT holds great promise as a therapeutic approach for AS. However, atherosclerotic lesions rebounded after a brief shrinkage of plaque following SDT in animal models due to the re-accumulation of lipids and macrophages in plaques. Retargeting macrophages through SDT can reverse this relapse [13]. Thus, multiple rounds of SDT may be required to sustain a curative effect and contribute to the successful control of AS.

Next, we will provide an overview of the effects and mechanisms of SDT on inflammatory cells, efferocytosis, lipids, smooth muscle cells (SMCs), matrix, endothelial cells and so on.

4.1. SDT ameliorates inflammation cells infiltration

The recognition of atherogenesis as an active process, rather than a passive cholesterol storage disease, has highlighted the role of inflammatory mechanisms. Both innate and adaptive immune responses contribute to the onset and progression of AS [38]. SDT reduced levels of pro-inflammatory cytokines interferon (IFN)-g, interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α while increasing secretion of anti-inflammatory cytokines transforming growth factor-β and IL-10. SDT transforms the pro-inflammatory environment of the atherosclerotic plaque into

Table 2
Application of sonodynamic therapy (SDT) in the clinical treatment of atherosclerosis.

Title	Application phase (recruitment status)	Research results	Clinical trial No./Ref.
Sonodynamic therapy on patients with femoropopliteal PAD and claudication	Phase I/II (withdrawn)	Not Applicable	NCT03318484
Sonodynamic therapy manipulates atherosclerosis regression trial on patients with carotid atherosclerotic plaques	Phase I/II (withdrawn)	Not Applicable	NCT03382249
Sonodynamic therapy manipulates atherosclerosis regression trial on patients with PAD and claudication	Phase I/II (completed)	Not Applicable	NCT03457662
Sonodynamic therapy in the treatment of carotid atherosclerosis	Phase I/II (completed)	Not Applicable	NCT03871725
Rapid inhibition of atherosclerotic plaque progression by sonodynamic therapy	Not applicable	The percentage of diameter stenosis decreased in PAD patients following combination therapy of atorvastatin and ALA-SDT.	[13]
Rapid reduction in plaque inflammation by sonodynamic therapy inpatients with symptomatic femoropopliteal PAD: A randomized controlled trial	Phase II	DVDMS-SDT rapidly reduced plaque inflammation and improved walking performance in PAD patients.	[19]
Sonodynamic therapy suppresses neovascularization in atherosclerotic plaques via macrophage apoptosis-induced endothelial cell apoptosis	Not applicable	DVDMS-SDT reduced neovascularization and arterial inflammation in PAD patients.	[21]

PAD: peripheral artery disease; ALA: aminolevulinic acid; DVDSM: sinoporphyrin sodium.

an anti-inflammatory one [13,25,32]. Several studies have verified SDT resolves inflammation via multiple mechanisms in AS. Here is a brief review of the effect of SDT on different type of inflammatory cells below.

4.1.1. SDT on macrophages

Macrophages are the predominant inflammatory cell type in atherosclerotic plaques, and they play a key role in the development and progression of the inflammatory response. Additionally, macrophages represent the main target cells for SDT in atherosclerotic plaques [13,35]. We will summarize the role and mechanism of SDT on macrophages in AS.

4.1.1.1. SDT-induced apoptosis in macrophages. In vitro and *in vivo* studies have reported that SDT can induce macrophage apoptosis using PpIX, ALA, HY, HSYA, Cur-ns and MALA: AuNPs as sonosensitizers. After SDT, macrophages exhibited typical apoptotic morphological changes, including the disappearance of cell microvilli, nuclear chromatin condensation on the nuclear envelope, and significant mitochondrial swelling with disappearing crista [23]. SDT induced macrophage apoptosis, reduced the release of TNF-α, IL-6 and IL-1β from macrophages, and alleviated inflammation response of advanced atherosclerotic plaque without off-target effects [13,29,31]. Sun et al. [13] found that caspase inhibitors reversed nearly all of the beneficial effects exerted by SDT, including inflammation regression, lipids decrease and efferocytosis increase. This suggests that SDT-induced apoptosis in lesional macrophages is the initial step driving subsequent beneficial events. In the following section, we will review the mechanism of macrophage apoptosis in detail. Cells undergo apoptosis through the mitochondrial-caspase apoptosis pathway [23]. When the mPTP opens, the mitochondrial transmembrane potential (ΔΨm) collapses due to the proton gradient dissipating in the mitochondrial intermembrane space. It has been reported that disruption of ΔΨm leads to the mechanical rupture of outer membrane, matrix swelling and release of intermembrane proteins including cytochrome C and apoptosis-inducing factor [39]. Once cytochrome C is released from the mitochondria into the cytoplasm, it activates the caspase-9 proenzyme, which in turn activates caspase-3. Caspase-3 cleaves poly (ADP-ribose) polymerase, initiating a caspase cascade that leads to cell apoptosis [23] (Fig. 2).

In 2015, Li et al. [23] reported that HY-SDT (intensity, 0.5 w/cm^2) induced apoptosis in THP-1 macrophages *in vitro* by targeting the mitochondria through ROS generation, which oxidizes mitochondrial membrane lipids. B cell lymphoma 2 (Bcl-2)-associated X protein (BAX) and cytochrome C subsequently translocated, and the translocation increased the ratio of the proapoptotic factor BAX to the antiapoptotic factor Bcl-2 in mitochondria. This causes mPTP opening, releasing cytochrome C to trigger the mitochondria−caspase pathway. In 2016, Zheng et al. [40] also indicated that HAC-SDT (intensity, 0.5 w/cm^2) generated ROS, promoted BAX and cytochrome C translocation and subsequently activated mitochondrial-caspase pathway. In 2018, Sun et al. [17] found ALA-SDT (intensity, 0.5 w/cm^2) activated TSPO to generate ROS, which leads to cytochrome C release from oxidated cardiolipins at the inner mitochondrial membrane and mPTP open at the outer mitochondrial membrane. This cytochrome *c* translocation in turn induced macrophages apoptosis through the mitochondria−caspase pathway. In 2019, Jiang et al. [29] reported that Cur-ns-SDT-induced macrophage apoptosis via generating ROS was regulated by cleaved caspase-9/3 via mitochondrial pathway, and the ultrasound intensity used was 0.4 w/cm^2. It is known that nuclear factor-kappaB (NF-κB) is the major inflammation-associated regulator nuclear factor. In 2020, Yao et al. [35] confirmed that ALA-SDT (intensity, 0.5 w/cm^2) induced macrophage mitochondrial apoptosis and reduced IL-1β, IL-6 and TNF-α through the ROS-peroxisome proliferator-activated receptor gamma (PPARγ)-NF-κB signaling pathway. In 2022, Cao et al. [37] deduced that DVDMS-SDT induced foam cell apoptosis via the mitochondria-caspase pathway. A commonality among the aforementioned studies is the use of SDT with an ultrasonic intensity not exceeding 0.5 w/cm^2 to induce macrophage apoptosis, thus providing further evidence supporting Yang et al. [5] and Sun et al. [41]'s perspective about ultrasound intensity Yang et al. [5] demonstrated that as ultrasound intensity increased beyond 0.8 w/cm^2, the necrotic ratio gradually increased as the ultrasonic intensity increased Sun et al. [41] found ALA-SDT (intensity, 0.48 and 0.84 w/cm^2) produced a small amount of ROS, mainly inducing apoptosis in THP-1 macrophages while SDT (1.16 w/cm2) produced a large amount of ROS, mainly inducing cell necrosis. However, the difference of ultrasonic intensity also may be attributed to variations in the type and dosage of sonosensitizers used, as well as differences in ultrasound exposure duration. Further investigations should prioritize these factors.

4.1.1.2. SDT-induced autophagy in macrophages. Autophagy refers to the degradation of various substrates in cells, such as lipids or damaged organelles, and contributes to cellular recovery. As a key

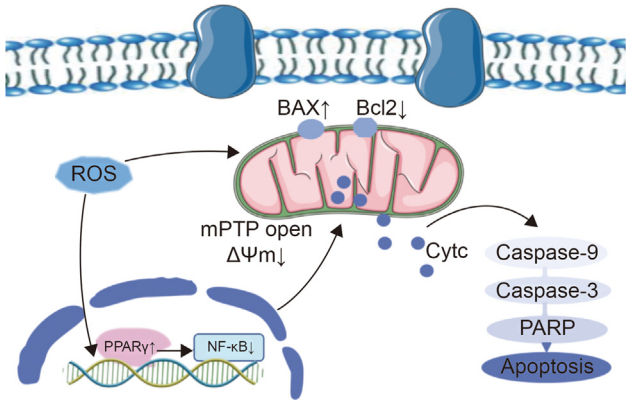

Fig. 2. Sonodynamic therapy (SDT) induces macrophage apoptosis. Reactive oxygen species (ROS) generated by SDT induce the opening of mitochondrial permeability transition pore (mPTP) and the loss of mitochondrial transmembrane potential (ΔΨm). Subsequently, cytochrome C (Cytc) is released from mitochondria into the cytosol to initiate a caspase cascade leading to apoptosis. SDT generates ROS to induce apoptosis via the peroxisome proliferator-activated receptor gamma-nuclear factor-kappaB (PPARγ-NF-κB) pathway. Bcl-2: B cell lymphoma 2; BAX: Bcl-2-associated X protein; PARP: poly (ADP-ribose) polymerase.

catabolic recycling pathway, autophagy plays vital roles in AS development. It is anti-apoptotic and maintains cellular homeostasis in adverse environment [24]. During early AS, autophagy at normal levels plays a role in atheroprotection; however, its function is impaired in advanced lesions. Notably, impaired macrophage autophagy results in decreased efferocytosis and hyperactivation of inflammasomes, thereby aggravating the progression of AS. Moreover, as autophagy is also highly related to lipid metabolism and cholesterol homeostasis, it has become a promising therapeutic mechanism for AS [41]. It has been found SDT plays a protective role in AS progression by modulating autophagy [24,40] (Fig. 3). In 2016, Zheng et al. [40] found that ROS produced by HAC-SDT (intensity, 0.5 w/cm^2) played a key role in initiating autophagy and apoptosis; while autophagy inhibited apoptosis, it effectively reduced lipid aggregation in macrophages. They demonstrated that autophagy was activated via phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mechanistic target of rapamycin (mTOR) pathway, a signaling pathway regulated by ROS generated during HAC-SDT. In the same year, Li et al. [24] reported that HY-SDT (0.25 μg/ml HY; intensity, 0.4 w/cm^2; exposure time, 15 min) induced AMP-activated protein kinase (AMPK)/AKT/mTOR pathway dependent autophagy and decreased lipid content in macrophages by regulating ROS-dependent transcription factor EB (TFEB) nuclear translocation from lysosome to nucleus to bind corresponding DNA sequences. Autophagy activation facilitates degradation of lipid droplet formed from engulfed oxidized low-density lipoprotein (ox-LDL) via cluster of differentiation 36 (CD36) and class A scavenger receptors (SR-A) into free fatty acid, promoting their efflux. Subsequently, several studies have also demonstrated the effect of non-lethal (NL)-SDT on autophagy in macrophages.

4.1.2. NL-SDT on macrophages

Considering that ultrasound intensity attenuates as sound waves pass through human tissue towards an atherosclerotic plaque and that macrophage survival state in human atherosclerotic plaques varies, the effects of very low-intensity SDT (NL-SDT) in AS should be assessed. In contrast to low-intensity SDT, NL-SDT does not induce macrophage cell apoptosis and necrosis [16]. The parameters of NL-SDT were determined by cell viability measured by flow cytometry or CCK-8 assays in distinct researches. In 2015, Sun et al. [41] found ultrasound (intensity, less than 0.48 w/cm^2) had no influence on ROS in macrophages and didn't induce apoptosis and necrosis. In 2017, Kou et al. [22] found cell viability decreased with an intensity of 0.6 w/cm^2 and above, but remained stable at 0.4 w/cm^2 as the exposure time of BBR-SDT increased. In the same year, Wang et al. [16] demonstrated that ALA-SDT (intensity, 0.1 and 0.4 w/cm^2) didn't induce apoptosis, but apoptotic cells increased when ultrasonic intensity rose to 0.8 w/cm^2. Jiang et al. [25] found cell viability was maintained until ultrasound irradiation of 10 min, intensity of 0.4 w/cm^2, and a concentration of HSYA at 0.6 mmol/L. Therefore, a cell viability assay should be conducted to obtain specific parameters for NL-SDT due to variations in sonosensitizer type and dosage, as well as ultrasound exposure time across different studies. Subsequently, further experiments can be initiated using the optimized parameters obtained from the previous trial. We will give a brief overview of the effect and mechanism of NL-SDT (Fig. 4).

4.1.2.1. NL-SDT induces autophagy and heme oxygenase 1 in macrophages. It has been observed that NL-SDT inhibits AS plaque progression by promoting ROS-dependent autophagy in macrophages [15,22,25]. In 2017, Kou et al. [22] demonstrated that NL-SDT (intensity, 0.4 w/cm2) with BBR as sonosensitizer produced ROS to induce autophagy via the PI3K/AKT/mTOR signaling pathway and subsequently promoted cholesterol efflux in macrophages and macrophage-derived foam cells. There exist certain similarities between Kou et al. [22] and Jiang et al. [25]. Jiang et al. [25] demonstrated that HSYA-SDT, using parameters capable of maintaining cell viability, induced an autophagic response through the suppression of PI3K/AKT/mTOR pathway and inhibited inflammation response by ROS in macrophages. In 2020, Yang et al. [15] showed NL-SDT (intensity: 0.1 w/cm^2; exposure time: 5 min) activated the ROS-AMPK-mechanistic target of rapamycin complex 1 (mTORC1)-autophagy pathway using ALA as sonosensitizer to enhance anti-inflammatory reactions, cholesterol efflux and proinflammatory macrophages (M1)-to-anti-inflammatory macrophages (M2) polarization *in vivo* and *in vitro*.

Heme oxygenase 1 (HO-1) is an inducible isoform of the heme oxygenase family that can break down heme into the ferrous iron, metabolites carbon monoxide and biliverdin. The metabolic products of HO-1 possess anti-inflammatory and antioxidant properties, and it has also been shown to protect against AS. HO-1 significantly reduces intraplaque hemorrhage (IPH) and increases plaque stability [42]. In 2017, Wcang et al. [16] verified that NL-SDT (intensity, 0.1 and 0.4 w/cm^2) with ALA as sonosensitizers inhibited the progression of atherosclerotic plaques, prevented ox-LDL-induced macrophage impairment, and reduced ox-LDL-induced ROS production through up-regulating macrophage expression of HO-1 in early atherosclerotic plaques. NL-SDT induced HO-1 expression by activating the nuclear factor erythroid 2-related factor 2 (Nrf2), AKT and extracellular signal-regulated kinase (ERK) pathways. Furthermore, the activation of signal transduction pathway changed over time, as indicated by the initiation of the ERK pathway 0.5 h after NL-SDT, followed by the AKT pathway at 1 h, and ultimately the Nrf2 pathway at 2 h post-treatment [16]. Their study confirmed for the first time that NL-SDT could induce HO-1 expression in ApoE$^{-/-}$ mice.

As mentioned above, autophagy has an anti-apoptotic effect. It has been reported that HO-1 also inhibits apoptosis [43]. Therefore, autophagy and HO-1 may be reasons why NL-SDT does not cause apoptosis.

4.1.2.2. NL-SDT enhances permeabilization of macrophages. When the incubation time is prolonged, the location of PpIX accumulation shifts from the initial plasma membrane to the cytoplasm. In 2019,

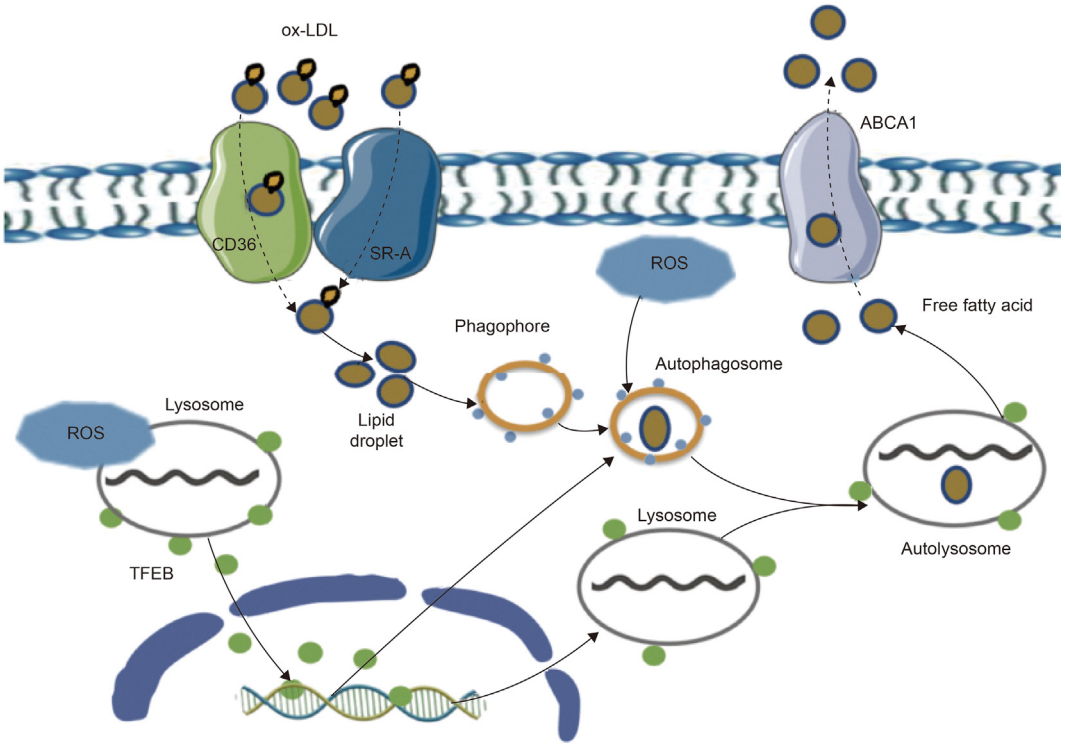

Fig. 3. The procedure of sonodynamic therapy (SDT) induces macrophage autophagy. Reactive oxygen species (ROS) generated by SDT regulates transcription factor EB (TFEB) to translocate from lysosome to nucleus, where it binds corresponding DNA sequences and subsequently triggers autophagy activation and lysosome regeneration. ox-LDL: oxidized low-density lipoprotein; CD36: differentiation 36; SR-A: class A scavenger receptors; ABCA1: ATP-binding membrane cassette transport protein A1.

Cao et al. [11] found that a 1-h incubation with 2 μM PpIX, ultrasonic intensity less than or equal to 0.4 w/cm^2 and an exposure time to 2 min didn't significantly reduce cell survival, indicating non-lethal for macrophages. Then, they applied a 1-h incubation of 2 μM PpIX followed by 0.4 w/cm^2 and 2 min of sonication as membrane-permeabilized SDT (MP-SDT). They found that when PpIX was mainly distributed on plasma membrane, MP-SDT could induce membrane deformation and permeabilization of macrophages via ROS generation, and this effect is better than ultrasound alone. Additionally, MP-SDT is a type of NL-SDT, which is milder than low-intensity SDT that induces cell apoptosis and is non-lethal for macrophages. Therefore, they believed that the membrane permeabilization might be reversible. They further confirmed that MP-SDT facilitated drug uptake in foam cells and enhanced the effect of atorvastatin by increasing the expression of PPARγ and ATP-binding cassette transporter G1 (ABCG1) as well as promoting cholesterol efflux. Their results suggested that MP-SDT holds promise as a way to improve the efficacy of anti-atherosclerotic drugs by facilitating drug delivery into intraplaque macrophages [11]. Moreover, modified membranes after binding with PpIX may make them more vulnerable and hypersensitive to ultrasound and mechanical stress, which could be potential mechanisms of MP-SDT. Therefore, the exact mechanisms of MP-SDT are complex and imprecise, and further investigation is still needed.

4.1.2.3. NL-SDT switches the phenotypic of macrophages. Activated macrophages are heterogeneous depending on the microenvironment and exhibit distinct pathologic roles through different subsets [44]. These activated macrophages are mainly classified into M1 or M2 subsets. M1 macrophages can increase the production of inflammatory mediators and tissue-degrading enzymes such as matrix metalloproteinases (MMPs). Thus, M1 contributes to sustained inflammation and plaque vulnerability [45]. While activated M2 macrophages resist cholesterol loading, store esterified cholesterol, and enhance collagen production to facilitate fibrous cap formation [46], the anti-inflammatory and profibrotic M2 phenotypes actively counteract M1-derived deleterious effects to stabilize plaques. The modulation of macrophage polarization has emerged as a novel therapeutic strategy for AS treatment.

In 2020, Jiang et al. [29] demonstrated that Cur-ns-SDT played an anti-AS role by promoting the transformation from M1 to M2 macrophages. Moreover, autophagy is a key mechanism for regulating macrophage polarization. Moderate activation of autophagy promotes polarization to M2 and alleviates inflammatory reactions [47]. Yang et al. [15] proposed that NL-SDT possessed the ability to mitigate inflammation and maintain cholesterol homeostasis, while promoting M1-to-M2 transition through activation of the ROS-AMPK-mTORC1-autophagy pathway in advanced atherosclerotic plaques, thereby significantly inhibiting AS progression. Therefore, phenotypic transformation of macrophages may be an important mechanism in the treatment of AS by NL-SDT.

Theoretically, NL-SDT may be a safer treatment option for severe plaques due to reduced susceptibility to complications from acute plaque rupture. NL-SDT could potentially become a new research hotspot in the future.

4.1.3. SDT on T lymphocytes

T lymphocytes, particularly CD4$^+$ T helper (Th) cells, are present within human plaques and have been identified as an important role in the regulation of inflammation during atherogenesis [48]. Based on their signature cytokine production and functional properties, activated Th cells can be classified into two main subsets: Th1 cells and Th2 cells [49]. The Th1-cell response, mediated by IFN-γ, produces pro-atherogenic inflammation mediators and activates

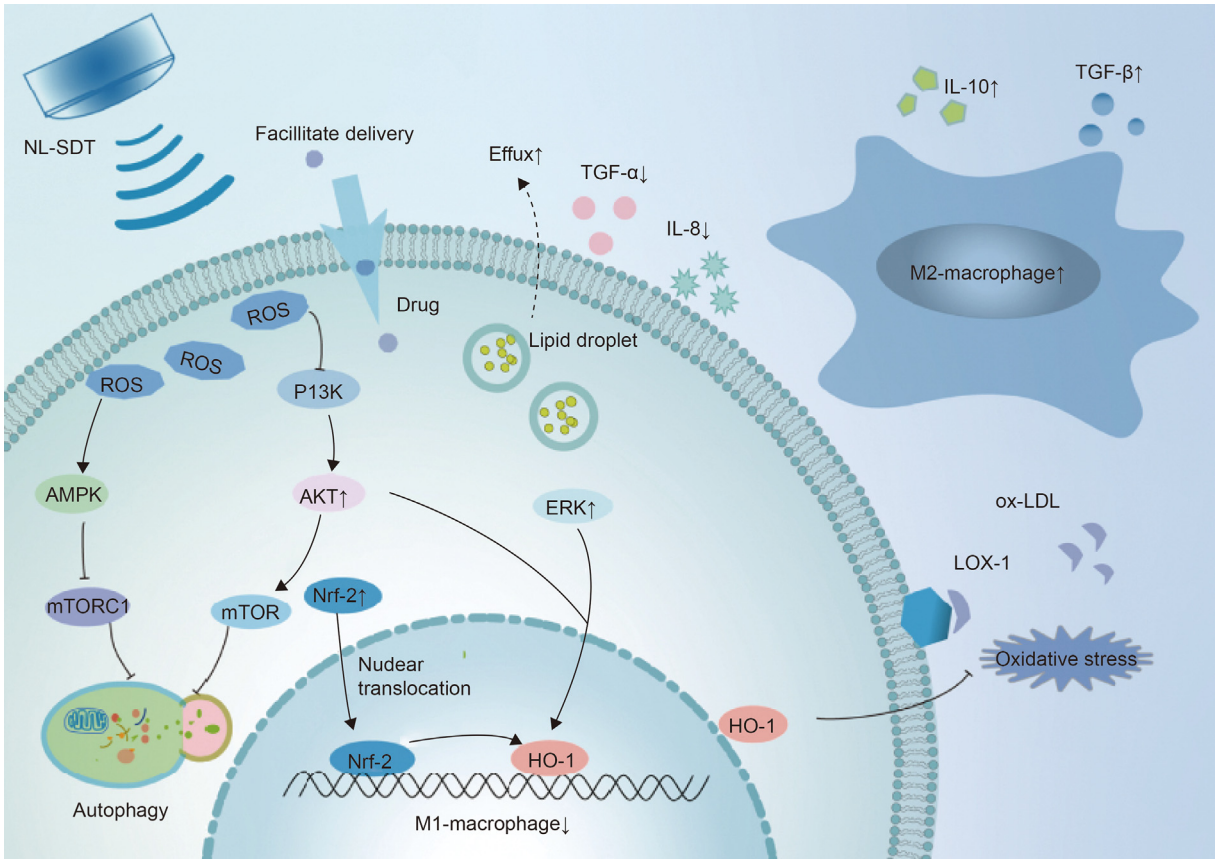

Fig. 4. The effect and mechanism of non-lethal-sonodynamic therapy (NL-SDT) in macrophages. NL-SDT induces autophagy via phosphoinositide 3-kinase/protein kinase B/mechanistic target of rapamycin (PI3K/AKT/mTOR) pathway to promote cholesterol efflux and inhibit inflammation by reactive oxygen species (ROS) in macrophages. NL-SDT activates the ROS-AMP-activated protein kinase (AMPK)-mechanistic target of rapamycin complex 1 (mTORC1)-autophagy pathway to enhance anti-inflammatory reactions, cholesterol efflux and proinflammatory macrophages (M1)-to-anti-inflammatory macrophages (M2) polarization. NL-SDT induces heme oxygenase 1 (HO-1) expression by activating the transcription factor nuclear factorerythroid 2-related factor 2 (Nrf2), and AKT and extracellular signal-regulated kinase (ERK) pathways to prevent oxidized low-density lipoprotein (ox-LDL)-induced macrophage impairment. Additionally, NL-SDT enhances macrophages permeabilization to facilitate drug delivery into macrophages. IL: interleukin; TGF-β: transforming growth factor-β; LOX-1: lectin-like oxidized low-density lipoprotein receptor-1.

macrophages, and thins fibrous cap by enhancing chemokine and protease secretion and degrading collagen [50]. Th2 cells release cytokines IL-10, IL-13 and IL-25 that suppress inflammation and AS [51]. Therefore, the balance between these Th1 and Th2 cell subsets may influence the development of AS, and increasing regulation of Th1/Th2 could provide a novel therapeutic strategy for AS.

In 2018, Yang et al. [5] demonstrated that 1 mM ALA with 0.8 w/cm^2 ultrasonic intensity was the optimal therapeutic parameters for inducing apoptosis. They showed that ALA-SDT efficiently ameliorates AS by activating the mitochondrial apoptotic signaling pathway to exert cytotoxic effects on Th cells, subsequently activating efferocytosis. Meanwhile, ALA-SDT induced a shift in the Th1/Th2 balance towards Th2 cells. The ultrasound field is heterogeneous, and ultrasonic waves attenuate through human tissue as they approach an atherosclerotic plaque. Consequently, cells at varying distances or depths are exposed to distinct ultrasound fields. Yang et al. [5] analyzed SDT with different energies generated different levels of ROS, thereby causing two distinct effects simultaneously, namely, pro-apoptotic effects and stimulation of Th1/Th2 shifting. This provides new insights into the mechanisms of SDT treatment as a novel therapeutic strategy for combating AS.

4.2. SDT enhances efferocytosis

Efferocytosis, also known as effective phagocytosis, is a crucial process in the early stages of AS. Following apoptosis, foam cells release “eat me” signals that are recognized by phagocytes [52]. However, the efferocytotic process is impaired in the advanced atherosclerotic plaques, resulting in insufficient clearance of apoptotic bodies and subsequent chronic release of inflammatory factors that lead to harmful secondary necrosis and inflammation response [53]. Thus, adequate phagocytosis of apoptotic cells by macrophages prevents necrotic core expansion and curbs AS progression. Several studies found that SDT induced foam cells and T cells apoptosis, as well as facilitated the recruitment of circulating monocytes into plaques. This subsequently increased efferocytosis to eliminate these apoptotic cells, thereby reducing the size of necrotic core and lesion [5,13,18,37]. In 2018, Wang et al. [18] demonstrated that ALA-SDT enhanced efferocytosis of foam cells following apoptosis via the PPARγ-liver X-receptor-alpha (LXRα) pathway, inducing the expression of the cell surface receptor Mer tyrosine kinase in advanced plaques. In 2022, Cao et al. [37] showed that DVDMS-SDT effectively promoted efferocytosis by activating caspase 3 to inhibit the antiphagocytic cell surface molecule, cluster of differentiation 47 (CD47), thereby reducing inflammation in advanced atherosclerotic plaques. In addition, it is known that DVDMS-SDT-activated caspase-3 cleaves specificity protein-1 (SP-1) and subsequently inhibits the transcription of hypoxia-inducible factor-1alpha (HIF-1α) in macrophage foam cells [54]. Therefore, it was postulated that DVDMS-SDT activated mitochondria-caspase apoptosis while activated caspase-3 enhances efferocytosis by repressing the expression of SP-1-HIF-1α-CD47 in lesional

macrophage. The hypothesis should be validated through future research endeavors.

4.3. SDT ameliorates lipid overload

Lipid metabolism disorder results in chronic inflammation of arterial wall, which eventually leads to AS. It has been reported that SDT may reduce the lipid-rich necrotic core area by decreasing lipid uptake and promoting lipolysis and lipid efflux [5,11,18,22,24] (Fig. 5). Macrophages is essential in regulating lipid metabolism. The reduction of lipids after SDT is attributed to the decrease of macrophages, which are mainly lipid-rich foam cells within plaques. Normally, ox-LDL is largely engulfed by scavenger receptors of macrophage and balanced by reverse cholesterol transporters [55]. However, macrophage metabolic capacity is impaired and cholesterol efflux decreases in advanced atherosclerotic plaques [56]. Overloaded lipids increase the formation of macrophage foam cells, and the incidence of plaque rupture and clinical complications [57]. Therefore, efficient lipids clearance is essential to prevent lipid buildup or foam cell formation, which is a promising strategy for AS treatment.

In 2016, Li et al. [24] demonstrated HY-SDT decreased lipid uptake, and enhanced lipid breakdown and efflux through autophagy activation via ROS-dependent TFEB nuclear translocation in macrophages. Additionally, ROS-dependent TFEB nuclear translocation increased the expression level of ATP-binding membrane cassette transport protein A1(ABCA1) to promote lipid efflux, and decreased the expression levels of cluster of to inhibit lipid uptake in macrophages. These results suggested that HY-SDT-activated TFEB nuclear translocation not only promoted lipolysis via lysosome regeneration and autophagy activation in macrophages, but also reversed cholesterol transport by decreasing lipid uptake and increasing lipid efflux. Consistent with Li's findings, Kou et al. [22] demonstrated that BBR-SDT induced autophagy to promote cholesterol efflux by increasing ROS generation in 2017. Additionally, the level of ABCA1 after BBR-SDT was also found to increase to promote lipid efflux. In 2018, Wang et al. [18] found ALA-SDT enhanced the cholesterol efflux and influx abilities of phagocytes in plaques, with the net effect of increasing lipid efflux. They demonstrated ALA-SDT induced activation of the PPARγ-LXRα-ABCA1/ABCG1 pathway to enhance cholesterol efflux in phagocytes and advanced plaque. In 2020, Yang et al. [15] found that NL-SDT inhibited AS progression by activating ROS-AMPK-mTORC1-autophagy pathway, resulting in a significant decrease in lipids. Therefore, SDT could efficiently remove lipids from atherosclerotic plaques and may be a promising strategy for treating AS.

4.4. SDT on neovascularization and vascular endothelial cells

Pathological angiogenesis is a major characteristic of vulnerable plaque [58]. The immature vascular plexus facilitates the recruitment of additional macrophages to the necrotic core, aggravating

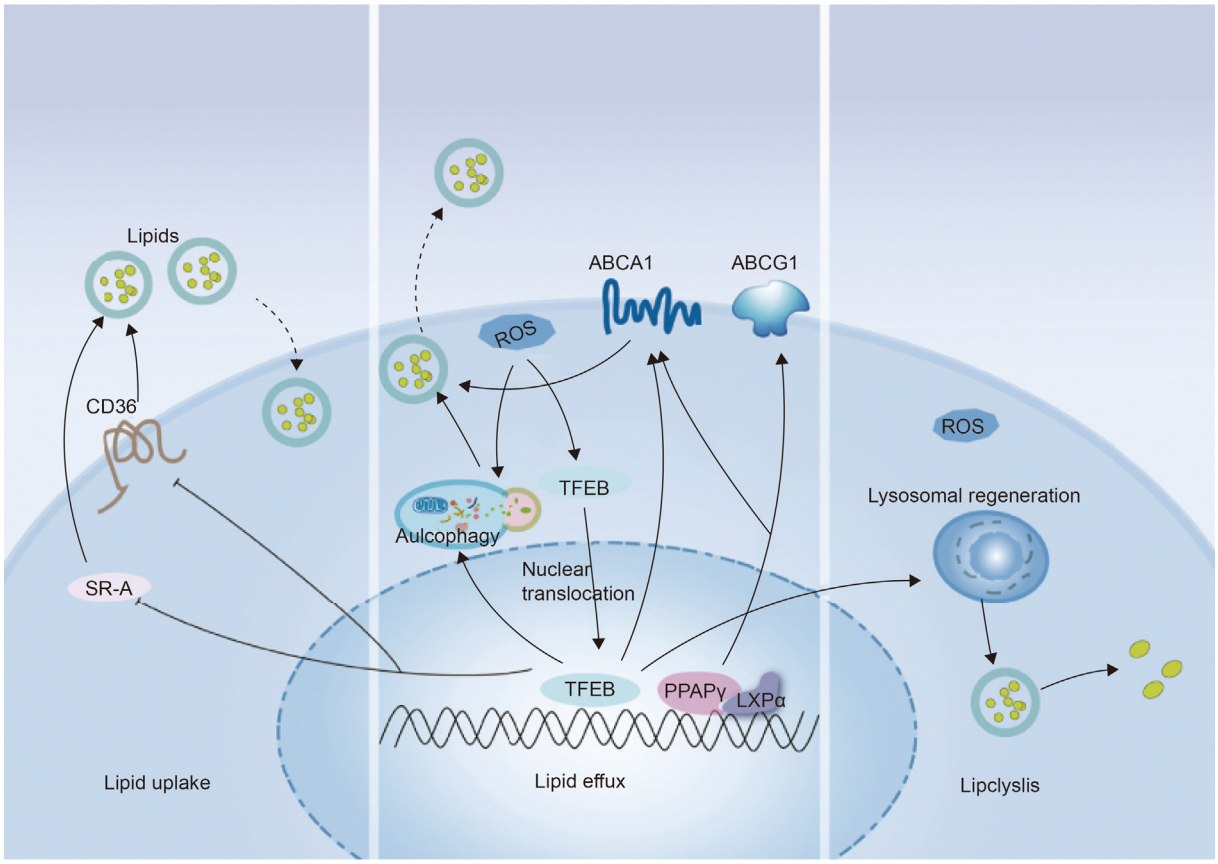

Fig. 5. Effect and mechanism of sonodynamic therapy (SDT) on lipids in atherosclerosis (AS). SDT decreases lipid uptake, enhances lipid breakdown and efflux in macrophage. transcription factor EB (TFEB) nuclear translocation triggered by SDT promotes autophagy activation and lysosome regeneration which enhances lipid degradation. Reactive oxygen species (ROS)-dependent TFEB nuclear translocation increases the expression level of ATP-binding membrane cassette transport protein A1 (ABCA1) to promote lipid efflux, and decreases the expression levels of differentiation 36 (CD36) and class A scavenger receptors (SR-A) to inhibit lipid uptake in macrophages. SDT induces the activation of peroxisome proliferator-activated receptor gamma-liver X-receptor-alpha-ATP-binding membrane cassette transport protein A1-ATP-binding cassette transporter G1 (PPARγ-LXRα-ABCA1/ABCG1) pathway, which enhances cholesterol efflux.

the cycle of inflammation present in atherosclerotic lesions [59]. Consequently, the inhibition of plaque neovascularization has become an important therapeutic strategy for reducing plaque rupture and secondary cardiovascular events. In 2020, it was found ALA-SDT could inhibit neointima formation without affecting re-endothelialization, reduce proliferating SMCs, macrophages and collagen but increase elastin in the plaque, and promote vascular blood perfusion [35]. In the same year, Yao et al. [21] found that DVDMS-SDT made advanced atherosclerotic plaque more stable by suppressing neovascularization in animal experiments. However, SDT had no direct effect on endothelial cells. This may be attributed to the exclusive uptake of DVDMS by macrophages rather than arterial endothelial cells. When SDT-treated macrophage foam cells were co-cultured with vascular endothelial cells, the apoptotic rate of endothelial cells increased dramatically under the influence of foam cell apoptosis. They demonstrated that DVDMS-SDT promoted apoptotic macrophage foam cell—induced neovascular endothelial cell apoptosis and inhibited proliferation, migration, and tubulogenesis of endothelial cell by reducing the levels of HIF-1α and vascular endothelial growth factor-A (VEGF-A) through caspase 3-SP-1—HIF-1 signaling pathway. Although vessel density reduces after DVDMS-SDT treatment, it remains unclear to what extent the remaining vessels are functional. Future research should focus on gaining further insights into these questions, as an increase in functional vessels may improve oxygen supply to the necrotic core and reduce oxidative stress, potentially breaking the vicious cycle of continuous macrophage recruitment. The authors also conducted a small size pilot study in patients, which demonstrated a reduction in plaque vessel density and inflammation measured by contrast-enhanced ultrasonography and fludeoxyglucose F18-PET/CT after DVDMS-SDT treatment [21]. However, the small-scale pilot study was neither double-blinded nor randomized. Further proper randomized clinical trials are necessary to facilitate the transition of SDT. Nonetheless, the clinical application and therapeutic effect were limited by the absence of a technique for real-time visualization of the distribution of sonosensitizers and SDT's inability to directly act on neovascular endothelial cells. In 2021, the developed sonosensitizer PHPMR NPs overcome these shortcomings. With excellent magnetic resonance imaging (MRI)/photoacoustic/ultrasound imaging ability, the distribution of PHPMR NPs in plaque can be in real-time observation. Additionally, PHPMR NPs with an anti-vascular endothelial growth factor receptor-2 antibody actively accumulated in the mitochondria of endothelial cells, and the PHPMR NPs-mediated SDT promoted mitochondrial-caspase apoptosis via the production of ROS and inhibited the proliferation, migration, and tubulogenesis of endothelial cells directly [32]. Moreover, Yao et al. [32] also suggested that targeted NPs-mediated SDT was superior to DVDMS-SDT in inhibiting plaque neovascularization, subsequently reducing IPH and inflammation, and ultimately stabilizing the plaque. PHPMR NPs-mediated SDT presents a safe and effective theranostic strategy for inhibiting plaque angiogenesis**.** It is worth noting that SDT does not induce any harm to the normal vascular endothelial cells [13,21,32].

Neovascularization rupture leads to IPH, which triggers plaque vulnerability and consequently causes acute cardiovascular events. The deposition of erythrocyte lysis products, which causes cholesterol deposits and iron retention in plaques, plays a key role in the aggressive progression of AS [60]. Iron-mediated oxidative injury further exacerbates the risk of plaque destabilization. In additional, intracellular iron overload strongly stimulates foam cell transformation and pro-inflammatory cytokine secretion by macrophages [61]. In 2020, Li et al. [20] revealed that DVDMS-SDT inhibited iron-overload-induced foam cell formation and proinflammatory cytokine secretion both *in vivo* and *in vitro* studies. Additionally, SDT reduced iron retention by stimulating ferroportin-1 expression through the ROS-Nrf2-ferroportin-1 (FPN1) pathway in macrophages. Therefore, SDT could potentially serve as a translational strategy for patients with hemorrhagic plaques in clinical practice.

4.5. SDT on SMCs and extracellular matrix

SMCs and extracellular matrix, including collagen and MMPs, are crucial in both mechanical stability and plaque growth. It is generally accepted that stable plaques are characterized with a thick fibrous cap that contains more SMCs and collagen mainly secreted by SMCs. It has been reported that the plaques treated with SDT contained fewer lipids and macrophages, but more SMCs and collagen with increased plaque fibrous cap thickness in advanced atherosclerotic lesions [12,13,15,20,32,37]. However, differently, Yao et al. [32] found that PHPMR NPs-mediated SDT increased collagen content but did not change SMCs content in the plaque, suggesting that SDT had no impact on collagen synthesis. After inhibition of neovessels by SDT, the number of macrophages entering the plaque through neovessels decreased significantly, and the expression and secretion of MMP-2 and MMP-9 also decreased, resulting in the reduction of collagen degradation.

Carotid artery stenting and percutaneous coronary intervention have been developed as routine treatments modalities. However, intimal hyperplasia is a major problem after these treatments, leading to vascular restenosis, renewed symptoms and the need for reoperation. Restenosis takes place in concert with the proliferation and migration of SMCs from the tunica media through the disruption of intima or endothelial barrier after intervention [62]. In a mature blood vessel, SMCs express smooth muscle-specific contractile markers and exhibit a differentiated phenotype with low proliferation and migration [63]. During the process of restenosis or early AS, SMCs transform from a differentiated phenotype to a dedifferentiated one characterized by expressing few contractile markers and high rates of proliferation, migration and synthesis of extracellular matrix components [64]. Dedifferentiated synthetic SMCs further migrate into subintima, hyperproliferate and secret large amounts of collagen, accompanied with the decrease of elastin, resulting in intima hyperplasia and luminal stenosis [65]. Phenotypic transformation of SMCs may provide a new strategy for the treatment of AS. In 2002, it was reported that SDT reduced the incidence of restenosis after iliac artery stenting in rabbits [66]. In 2015, it was shown that ALA-SDT promoted the transformation of the SMCs phenotype from the dedifferentiated to differentiated status via ROS and activated p38 mitogen-activated protein kinase [67]. In 2017, Li et al. [10] demonstrated that PpIX-SDT did not induce apoptosis but increased autophagy in SMCs. Autophagy is crucial for normal function, phenotype, and survival of SMCs. However, the molecular mechanisms by which SDT activates autophagy in SMCs remain to be further clarified. In 2020, Yao et al. [35] found that ALA-SDT significantly inhibited intimal hyperplasia by decreasing macrophages, proliferative SMCs, and collagen content but while increasing elastin content in early balloon-injured arteries of hypercholesterolemic rabbits without influencing endothelium regeneration, but ALA-SDT had no direct effect on human umbilical artery SMCs (HUASMCs), as HUASMCs uptake sonosensitizers much slower than macrophages. However, when THP-1-derived macrophages were co-cultured with TNF-α-stimulated HUASMCs, ALA-SDT-treated THP-1-derived macrophages remarkably inhibited HUASMC proliferation, migration and IL-6 production. Since inflammatory cytokines are widely recognized to activate SMCs, it was inferred that decreased IL-1β, IL-6 and TNF-α in macrophages induced by ALA-SDT indirectly inhibited proliferation and migration of HUASMCs. Furthermore, they discovered

that early intervention with ALA-SDT may efficiently inhibit intimal hyperplasia while not affecting re-endothelialization [35]. However, the underlying interaction between macrophages and SMCs after ALA-SDT still requires further clarification.

MMPs, mainly produced by macrophages, degrade extracellular matrix proteins [68], leading to plaque vulnerability. It has been observed that ALA-SDT induced apoptosis of lesional macrophages decreased the production of MMP-2 and -9, which can degrade collagen and result in thinning of the fibrous cap [12]. It is believed that reducing MMPs levels enhances collagen levels and stabilizes plaque, thereby increasing SDT's long-term efficiency [13]. In 2022, Tian et al. [69] observed that DVDMS-SDT effectively inhibited matrix collagen degradation in advanced atherosclerotic plaque by modulating caspase 3-pig-ment epithelium-derived factor(caspase 3-PEDF)/HIF-1α-MMP-2/MMP-9 signaling to reduce the secretion and expression of MMP-9 and MMP-2 in macrophage foam cells. Therefore, it represents a suitable and promising clinical regimen for stabilizing vulnerable plaques.

5. The safety of SDT

SDT displays strength in its safety profile. The SDT procedure is non-invasive which avoids procedure-related risks associated with operative treatments. Additionally, some sonosensitizers selectively accumulate in macrophages or endothelial cells and atherosclerotic plaques, but they cannot exert effects without ultrasound [6,32]. Due to the characteristics of local treatment of SDT, off-target and systemic side effects are avoided [18].

Sun et al. [13] observed that neither plaque rupture nor permanent muscle damage occurred at the SDT-irradiated site, and SDT did not cause the death of any experimental animals, including mice and rabbits. Yao et al. [21] found that DVDMS didn't accumulate in normal femoral arteries or arterial endothelial cells in animal models, and SDT didn't alter the proliferation, migration, tubulogenesis and viability of human umbilical vein endothelial cells. In other words, DVDMS-SDT does not cause damage to normal vascular endothelial cells. Yao et al. [32] also demonstrated that PHPMR NPs-mediated SDT had no effect on the coverage and function of endothelial cells in rabbits. Additionally, there were no significantly differences in blood biochemical indices and weight fluctuations compared to the control group, and histopathological examination of major organs indicated undetectable toxicity after SDT in rabbits. These results suggest the high therapeutic biosafety by PHPMR NPs-mediated SDT in combating plaque neovascularization. In 2021, a randomized controlled clinical trial showed that DVDMS-SDT did not cause any plaque rupture as assessed by diagnostic ultrasound. And it had no impact on normal immunocytes, as evidenced by the absence of changes in circulating leucocytes or monocyte counts. This finding is consistent with the specific targeting effect of DVDMS-SDT on plaque

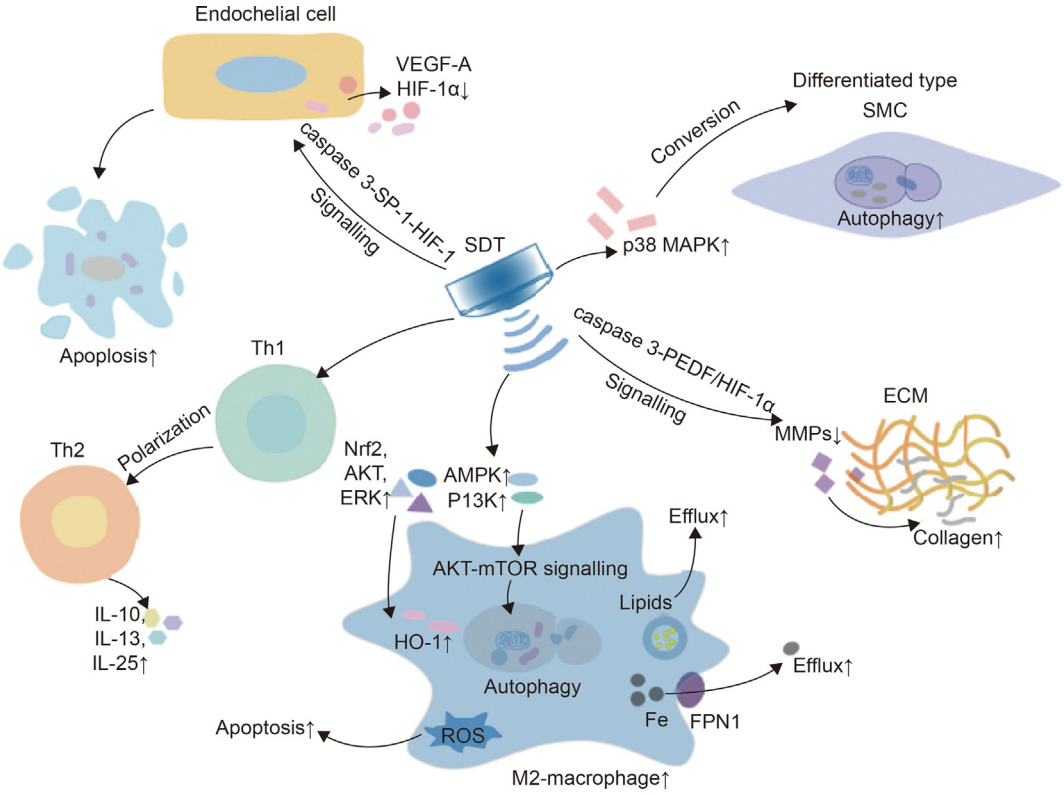

Fig. 6. The effect and mechanism of sonodynamic therapy (SDT) on atherosclerosis. SDT induces macrophage mitochondrial apoptosis through reactive oxygen species-peroxisome proliferator-activated receptor gamma-nuclear factor-kappaB (ROS-PPARγ-NF-κB) signaling pathway, subsequently increasing efferocytosis to eliminate these apoptotic cells. SDT induces AMP-activated protein kinase/protein kinase B/mechanistic target of rapamycin (AMPK/AKT/mTOR) pathway dependent autophagy by regulating ROS-dependent transcription factor EB (TFEB) nuclear translocation via and PI3K/AKT/mTOR pathway. Non-lethal (NL)-SDT induces heme oxygenase 1 (HO-1) expression by activating the transcription factor nuclear factorerythroid 2-related factor 2 (Nrf2), AKT and extracellular signal-regulated kinase (ERK) pathways. SDT reduces iron retention by stimulating ferroportin-1 (FPN1) expression through ROS-Nrf2-FPN1 pathway in macrophages. SDT induces a shift in the T helper (Th)1/Th2 balance towards Th2 cells. SDT promotes apoptotic macrophage foam cell-induced neovascular endothelial cell apoptosis and inhibits proliferation, migration, and tubulogenesis of endothelial cell by reducing the levels of hypoxia-inducible factor-1alpha (HIF-1α) and vascular endothelial growth factor-A (VEGF-A) through caspase 3-specificity protein-1 (SP-1)-HIF-1 signaling pathway. SDT promotes the transformation of smooth muscle cell (SMC) phenotype from the dedifferentiated to differentiated status via ROS and activated p38 mitogen-activated protein kinases (MAPK). SDT increases autophagy in SMC. SDT inhibits matrix collagen degradation in advanced atherosclerotic plaque by modulating caspase 3-pig-ment epithelium-derived factor (PEDF)/HIF-1α–matrix metalloproteinase (MMP)-2/MMP-9 signaling to reduce the secretion and expression of MMP-9 and MMP-2 in macrophage foam cells. ECM: extracellular matrix; IL: interleukin.

macrophages. It has been asserted that SDT demonstrates high efficacy with minimal side effects in animal models and clinical patients of AS [19]. In the future, it will be necessary to conduct a large clinical trial to confirm the efficacy and safety of SDT.

6. Conclusion

To sum up, SDT can decrease the percentage of lumen stenosis and stabilize the plaque by inducing macrophages and T cells apoptosis, macrophages and SMCs autophagy, switching the phenotypic of macrophages, T cells and SMCs, inhibiting neovascularization formation, activating efferocytosis, enhancing collagen, reducing MMPs levels and so on. These effects collectively contribute to its anti-atherosclerotic properties (Fig. 6). The mechanism underlying plaque inhibition is still under constant exploration. Clinical randomized controlled trials have already been carried out with initial positive results in alleviating AS [13,19,21]. However, multiple rounds may be necessary to sustain plaques regression and successfully control AS [13]. Further studies should be carried out using repeated SDT to prevent rebound and better inhibit plaque development. Additionally, the development of targeted nanosonosensitizer enables real-time monitoring of the sonosensitizers distribution at the molecular level, making SDT more accurate and efficient.

The findings of NL-SDT suggested that different ultrasonic parameters of SDT exert equally beneficial effects locally through divergent mechanisms. This phenomenon may be attributed to the varying energies of SDT, resulting in distinct ROS levels and simultaneous diverse effects. Besides, the ultrasound field itself is heterogeneous. Moreover, when propagating inside human body, ultrasonic waves can be reflected and the ultrasonic energy attenuated through a medium and human tissue towards an atherosclerotic plaque. Therefore, even SDT with the same parameters, cells at different depths are also exposed to varying ultrasound fields. Hence, exploring ROS levels induced by SDT with different ultrasonic parameters and investigating biological effects and mechanisms associated with different levels of ROS could be highly significant for future research and translational applications.

CRediT author statement

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Declaration of competing interest

The authors declare that there are no conflicts of interest.

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