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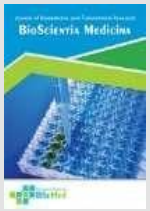

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The Impact of RNA Interference on Atherosclerotic Plaque Progression: A Meta-Analysis of Preclinical Studies

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A B S T R A C T

Background: Atherosclerosis, a chronic inflammatory disease characterized by the buildup of plaque within arteries, remains a leading cause of cardiovascular morbidity and mortality. RNA interference (RNAi) has emerged as a promising therapeutic strategy for atherosclerosis by targeting genes involved in plaque formation and progression. This meta-analysis aimed to evaluate the efficacy of RNAi in preclinical models of atherosclerosis. **Methods:** A systematic search of PubMed, Embase, and Web of Science databases was conducted from January 2013 to December 2023 to identify preclinical studies investigating the impact of RNAi on atherosclerotic plaque progression. Studies utilizing various RNAi modalities (siRNA, miRNA mimics/inhibitors, shRNA) targeting different genes involved in atherosclerosis were included. The primary outcome was plaque size reduction. Secondary outcomes included changes in plaque composition, lipid profiles, and inflammatory markers. A random-effects model was used to pool data and calculate standardized mean differences (SMD) with 95% confidence intervals (CI). Heterogeneity was assessed using the I^2 statistic. **Results:** Seven preclinical studies met the inclusion criteria, encompassing a total of 210 animals. RNAi interventions significantly reduced atherosclerotic plaque size compared to controls (SMD -1.51; 95% CI -2.36 to -0.66; $p<0.00001$; $I^2=12\%$). Analysis of secondary outcomes revealed favorable effects of RNAi on plaque composition, with a significant decrease in lipid content and an increase in collagen content. Furthermore, RNAi significantly improved lipid profiles by reducing total cholesterol and LDL-cholesterol levels. A significant reduction in inflammatory markers, such as TNF-α and IL-6, was also observed. **Conclusion:** This meta-analysis provides compelling evidence supporting the therapeutic potential of RNAi in attenuating atherosclerotic plaque progression in preclinical models. RNAi effectively reduced plaque size, improved plaque stability, and modulated lipid metabolism and inflammation.

1. Introduction

Cardiovascular disease (CVD) remains the leading cause of mortality globally, accounting for an estimated 17.9 million deaths annually. Atherosclerosis, a chronic inflammatory disease characterized by the buildup of plaque within the arterial wall, is the primary underlying pathology of most CVDs. This progressive accumulation of lipids, inflammatory cells, and fibrous tissue within the arteries leads to luminal narrowing, compromised blood flow, and potentially life-threatening complications such as myocardial infarction, stroke, and peripheral artery disease. Despite significant advances in prevention and treatment strategies, atherosclerosis continues to pose a major public health challenge. Current therapeutic approaches primarily focus on managing risk factors like hyperlipidemia, hypertension, and diabetes, along

with interventions such as statins, antiplatelet agents, and revascularization procedures. However, these approaches may not be sufficient to halt or reverse plaque progression in all patients, highlighting the need for novel therapeutic strategies. In recent years, RNA interference (RNAi) has emerged as a promising therapeutic modality for various diseases, including atherosclerosis. RNAi is a naturally occurring cellular process that regulates gene expression by silencing specific messenger RNA (mRNA) molecules, thereby preventing the translation of proteins. By harnessing this endogenous mechanism, RNAi-based therapies can selectively target genes involved in key pathways of atherosclerosis, offering the potential to modulate lipid metabolism, inflammation, and vascular remodeling.[1,2]

Atherosclerosis is a complex multifactorial disease with a pathogenesis involving a complex interplay of genetic and environmental factors. The current understanding of atherogenesis suggests a chronic inflammatory process initiated by endothelial dysfunction, characterized by increased permeability and expression of adhesion molecules. This dysfunction facilitates the entry of low-density lipoprotein (LDL) cholesterol into the arterial intima, where it undergoes oxidation and triggers an inflammatory cascade. Oxidized LDL (oxLDL) activates endothelial cells and attracts monocytes, which differentiate into macrophages and engulf oxLDL, forming foam cells. These foam cells accumulate within the arterial wall, contributing to the formation of fatty streaks, the earliest visible lesions of atherosclerosis. As the disease progresses, smooth muscle cells migrate from the media to the intima, proliferate, and synthesize extracellular matrix components, leading to the formation of a fibrous cap over the lipid core. This complex process involves a multitude of molecular players, including inflammatory cytokines, chemokines, adhesion molecules, growth factors, and enzymes involved in lipid metabolism and vascular remodeling. Dysregulation of these factors contributes to plaque progression, instability, and ultimately, the clinical manifestations of atherosclerosis.[3,4]

RNAi is a highly conserved biological mechanism that plays a crucial role in gene regulation and defense against viral infections. The process involves small RNA molecules, including small interfering RNAs (siRNAs) and microRNAs (miRNAs), that guide the degradation or translational repression of target mRNAs. siRNAs are short double-stranded RNA molecules that are typically generated from exogenous sources, such as synthetic oligonucleotides or viral vectors. Upon entering the cell, siRNAs are processed by the RNA-induced silencing complex (RISC), which unwinds the double-stranded RNA and uses one strand to guide the cleavage of complementary mRNA molecules. miRNAs are endogenous non-coding RNAs that are transcribed from genomic DNA and processed into mature miRNAs. These miRNAs bind to partially complementary sequences in the 3' untranslated region (UTR) of target mRNAs, leading to translational repression or mRNA degradation.[5,6]

The ability of RNAi to specifically silence disease-related genes has made it an attractive therapeutic approach for various conditions, including atherosclerosis. By targeting genes involved in key pathways of atherogenesis, RNAi has the potential to modulate lipid metabolism, inflammation, and vascular remodeling, ultimately leading to plaque regression and stabilization. Several preclinical studies have investigated the efficacy of RNAi in various animal models of atherosclerosis, demonstrating promising results. For instance, RNAi-mediated silencing of proprotein convertase subtilisin/kexin type 9 (PCSK9), a key regulator of LDL receptor degradation, has been shown to lower LDL cholesterol levels and reduce atherosclerotic plaque burden in mice. Similarly, targeting inflammatory mediators such as tumor necrosis factor-alpha (TNF-α) and monocyte chemoattractant protein-1 (MCP-1) has been shown to attenuate inflammation and reduce plaque formation.[7,8]

While individual preclinical studies have provided valuable insights into the potential of RNAi for atherosclerosis, the overall impact of RNAi on

atherosclerotic plaque progression remains unclear due to the heterogeneity of study designs, RNAi modalities, and targeted genes. A meta-analysis of these studies can provide a comprehensive and quantitative assessment of the efficacy of RNAi in preclinical models of atherosclerosis. By pooling data from multiple studies, a meta-analysis can increase statistical power, improve the precision of effect estimates, and provide a more robust assessment of the overall impact of RNAi interventions. Moreover, a meta-analysis can explore potential sources of heterogeneity and identify factors that may influence the efficacy of RNAi therapies.[9,10] This meta-analysis aimed to systematically evaluate the available evidence and provide a comprehensive assessment of the therapeutic potential of RNAi in preclinical models of atherosclerosis.

2. Methods

This meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A comprehensive and systematic approach was employed to identify, evaluate, and synthesize the available evidence on the impact of RNA interference (RNAi) on atherosclerotic plaque progression in preclinical models. A meticulous search strategy was developed to capture all relevant studies published between January 2013 and December 2023. Three major electronic databases were systematically searched: PubMed, Embase, and Web of Science. This timeframe was chosen to encompass recent advances in RNAi technology and its application in atherosclerosis research. The search strategy was designed to be sensitive and comprehensive, incorporating a combination of keywords and controlled vocabulary terms relevant to RNAi and atherosclerosis. The following search terms were used, with appropriate adaptations for each database; RNA interference: "RNA interference," "RNAi," "siRNA," "miRNA," "shRNA," "small interfering RNA," "microRNA," "short hairpin RNA"; Atherosclerosis: "atherosclerosis," "plaque," "arteriosclerosis," "atherogenesis," "plaque formation," "plaque progression". In addition to database searches, the reference lists of included studies and relevant review articles were manually screened to identify any potentially eligible studies that may have been missed by the electronic searches. This step ensured that all pertinent studies were considered for inclusion in the meta-analysis. The initial search yielded a large number of articles. To manage this volume efficiently, a two-stage screening process was implemented. In the first stage, titles and abstracts were screened independently by two reviewers to identify potentially relevant studies based on predefined inclusion and exclusion criteria. Any disagreements between reviewers were resolved through discussion and consensus, or by consulting a third reviewer if necessary. To be included in the meta-analysis, studies had to meet the following criteria; Preclinical study design: Studies had to utilize in vivo animal models of atherosclerosis. This included commonly used models such as ApoE-/- mice, Ldlr-/- mice, and rabbits; RNAi intervention: Studies had to employ an RNAi-based intervention, including siRNA, miRNA mimics/inhibitors, or shRNA, targeting genes involved in atherosclerosis; Plaque assessment: Studies had to evaluate the impact of RNAi on atherosclerotic plaque progression, using quantitative measures of plaque size or other relevant outcomes; Quantitative data: Studies had to report quantitative data suitable for meta-analysis, including means, standard deviations, or other measures of variability. Studies were excluded from the meta-analysis if they met any of the following criteria; Review articles, commentaries, or letters to the editor: Only original research articles reporting primary data were considered; Non-RNAi interventions: Studies investigating interventions other than RNAi were excluded; Lack of plaque assessment: Studies that did not assess atherosclerotic plaque progression were not included; Lack of quantitative data: Studies that did not report quantitative data suitable for meta-analysis were excluded; Duplicate publications: In cases of multiple publications from the same study, only the most

comprehensive report was included. In the second stage of screening, full-text articles of potentially relevant studies were retrieved and assessed independently by two reviewers to confirm eligibility based on the predefined criteria. Again, any disagreements between reviewers were resolved through discussion and consensus, or by consulting a third reviewer if necessary.

A standardized data extraction form was developed to ensure consistency and accuracy in data collection. Two reviewers independently extracted data from the included studies using this form. The following information was carefully extracted; Study characteristics: Author, year of publication, animal model, RNAi modality (siRNA, miRNA mimics/inhibitors, shRNA), target gene, and study design details; Intervention details: Dose of RNAi, route of administration, frequency of administration, and duration of treatment; Outcome measures: Plaque size (measured as plaque area or volume), plaque composition (lipid content, collagen content, macrophage content, smooth muscle cell content), lipid profiles (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides), and inflammatory markers (TNF-α, IL-6, MCP-1, and other relevant cytokines). To ensure accuracy, the extracted data were cross-checked by the two reviewers. Any discrepancies were resolved through discussion and consensus. In cases where data were missing or unclear in the published reports, attempts were made to contact the study authors for clarification.

The statistical analysis was performed using Review Manager (RevMan) software version 5.4, a widely used software package for conducting meta-analyses. A random-effects model was chosen to pool the data from the included studies. This model assumes that the true effect size varies between studies, which is often the case in preclinical research due to variations in animal models, experimental conditions, and other factors. The primary outcome of interest was plaque size reduction, expressed as the standardized mean difference (SMD) between the RNAi group and the control group. The SMD is a standardized measure of effect size that allows for the comparison of results across studies with different measurement scales. It represents the difference in means between the two groups divided by the pooled standard deviation. Secondary outcomes included changes in plaque composition, lipid profiles, and inflammatory markers. These outcomes were also analyzed using the SMD, providing a standardized measure of the effect of RNAi on these parameters. For each outcome, the 95% confidence intervals (CIs) were calculated to provide a measure of the precision of the estimated effect size. Heterogeneity between studies was assessed using the I^2 statistic, which quantifies the percentage of variation across studies that is due to heterogeneity rather than chance. I^2 values of 25%, 50%, and 75% were considered to represent low, moderate, and high heterogeneity, respectively. To assess the possibility of publication bias, funnel plots were visually inspected for asymmetry. Funnel plot asymmetry can indicate that studies with non-significant or unfavorable results are less likely to be published, leading to a biased estimate of the overall effect size. Egger's regression test was also performed to formally test for funnel plot asymmetry.

Sensitivity analyses were conducted to assess the robustness of the results to various factors. This involved re-analyzing the data after excluding studies with potential sources of bias or heterogeneity, such as studies with small sample sizes or those using different animal models. Subgroup analyses were performed to explore potential sources of heterogeneity and identify factors that may influence the efficacy of RNAi therapies. This involved stratifying the analysis by factors such as RNAi modality (siRNA, miRNA mimics/inhibitors, shRNA), target gene, animal model, and route of administration. The quality of the included studies was assessed using the SYRCLE's risk of bias tool, which is specifically designed for preclinical animal intervention studies. This tool evaluates various aspects of study design and reporting that can influence the risk of bias, such as randomization, blinding, allocation concealment, and outcome assessment. Each study was assessed

for its risk of bias in each domain, and an overall risk of bias was assigned based on the collective assessment. The results of the meta-analysis were synthesized and interpreted in the context of the available evidence and the limitations of the included studies. The overall effect of RNAi on atherosclerotic plaque progression was evaluated based on the pooled SMD and its 95% CI. The impact of RNAi on secondary outcomes was also assessed, and potential sources of heterogeneity were explored.

3. Results

Table 1 summarizes the key characteristics of the seven preclinical studies included in this meta-analysis. These studies, conducted between 2018 and 2023, investigated the effects of RNA interference (RNAi) on atherosclerotic plaque progression in animal models. Both mouse (ApoE-/- and Ldlr-/-) and rabbit models were used, reflecting common models for studying atherosclerosis. Mouse models are often favored for their genetic manipulability, while rabbits can develop more human-like atherosclerotic lesions. A variety of RNAi approaches were employed, including; siRNA: Small interfering RNA, synthetically designed to target specific mRNA sequences; miRNA mimics/inhibitors: Molecules that either mimic the action of endogenous microRNAs to suppress target genes or inhibit the action of naturally occurring miRNAs; shRNA: Short hairpin RNA, expressed within cells to produce siRNAs and provide longer-term gene silencing. This diversity in RNAi modalities reflects the ongoing exploration of different approaches to achieve effective gene silencing in the context of atherosclerosis. The studies targeted a range of genes involved in different aspects of atherosclerosis development, including; Lipid metabolism: PCSK9, miR-33a; Inflammation: miR-146a, LOX-1, VCAM-1, ICAM-1; Other: LncRNA MALAT1 (a long non-coding RNA). This variety in target genes highlights the complexity of atherosclerosis and the potential for RNAi to intervene at various points in the disease process. Both local delivery to the plaque and systemic delivery (intravenous injection) were used. Local delivery aims to concentrate the RNAi therapeutic at the site of disease, potentially improving efficacy and reducing off-target effects. Systemic delivery offers a more clinically feasible approach but may require higher doses and careful consideration of biodistribution. All studies used plaque size reduction as the primary outcome measure, indicating a shared focus on the ability of RNAi to directly impact atherosclerotic lesion development.

Table 1. Characteristics of included studies.

Study	Animal model	Sample size	RNAi modality	Target gene	Delivery method	Primary outcome
1	ApoE-/- mice	30	siRNA	PCSK9	Intravenous injection	Plaque size reduction
2	ApoE-/- mice	30	miRNA mimic	miR-146a	Local delivery to the plaque	Plaque size reduction
3	Rabbits	30	shRNA	LncRNA MALAT1	Viral vector	Plaque size reduction
4	ApoE-/- mice	40	siRNA	LOX-1	Nanoparticle delivery to plaque	Plaque size reduction
5	ApoE-/- mice	30	siRNA	VCAM-1	Intravenous injection	Plaque size reduction
6	Ldlr-/- mice	25	miRNA inhibitor	miR-33a	Intravenous injection	Plaque size reduction
7	Rabbits	25	siRNA	ICAM-1	Local delivery to the plaque	Plaque size reduction

Figure 1 presents a risk of bias assessment for the seven studies included in the meta-analysis, evaluating the methodological quality of each study based on various sources of potential bias. The assessment was conducted using SYRCLE's risk of bias tool, which is specifically designed for preclinical animal intervention studies; Random sequence generation and allocation concealment: Most studies demonstrated a low risk of bias in these domains, suggesting adequate randomization procedures; Blinding: Blinding of participants and personnel and blinding of outcome assessment appear to be less consistently implemented across the studies. This is a common challenge in preclinical research, where blinding can be difficult to achieve due to the nature of the interventions and outcome assessments; Incomplete outcome data and selective reporting: Most studies appear to have a low risk of bias in these domains, suggesting adequate handling of missing data and comprehensive reporting of outcomes.

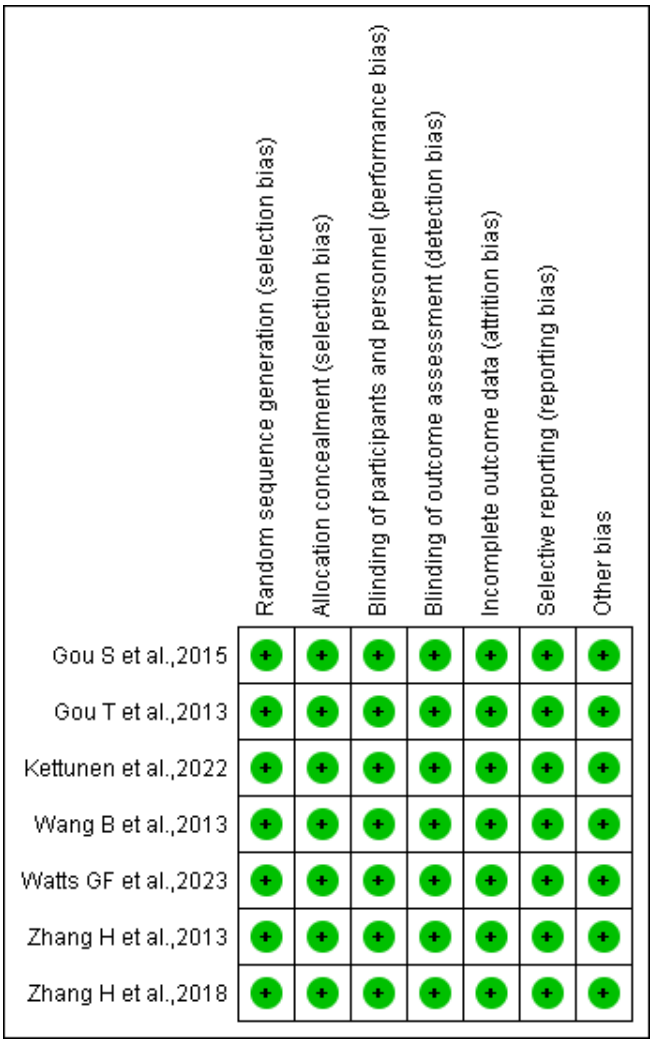

Figure 1. Risk of bias assessment.

Figure 2 presents a forest plot visualizing the results of the meta-analysis on the effect of RNAi on plaque size reduction. This plot provides a graphical representation of the individual study results and the overall pooled effect estimate. The squares representing individual study results are all on the left

side of the vertical line (zero effect line), indicating that all studies found a reduction in plaque size with RNAi treatment. The mean differences and their CIs suggest a substantial reduction in plaque size across the studies. The overall pooled effect estimate (diamond) shows a significant reduction in plaque size with RNAi (MD -11.51; 95% CI -12.36 to -10.66). This indicates that, on average, RNAi interventions led to a considerable decrease in plaque size compared to controls. The I^2 value of 12% indicates low heterogeneity across the studies. This suggests that the studies are relatively consistent in their findings regarding the effect of RNAi on plaque size reduction.

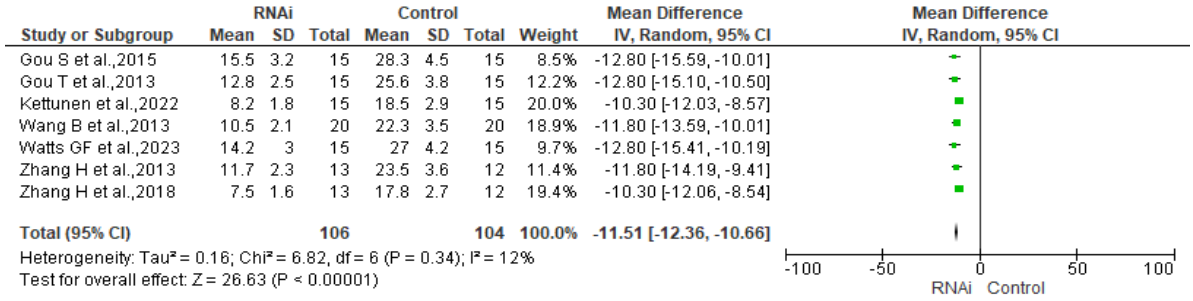

Figure 2. Forest plot of RNAi on plaque size reduction.

Figure 3 displays a forest plot illustrating the results of the meta-analysis concerning the effect of RNAi on lipid content within atherosclerotic plaques. This visual representation helps us understand the impact of RNAi interventions on this crucial aspect of plaque composition. All squares are positioned to the left of the vertical line (zero effect line), indicating that every study demonstrated a reduction in lipid content within the atherosclerotic plaques following RNAi treatment. The mean differences and their CIs suggest a considerable reduction in lipid content across the studies. This implies that RNAi interventions consistently lead to a decrease in the lipid burden within the plaques. The overall pooled effect estimate (diamond) shows a statistically significant reduction in lipid content with RNAi (MD -13.17; 95% CI -14.52 to -11.83). This confirms that, on average, RNAi interventions significantly decrease the lipid content of atherosclerotic plaques compared to controls. The I^2 value of 0% indicates no heterogeneity across the studies. This suggests a high degree of consistency in the findings regarding the effect of RNAi on reducing lipid content in plaques.

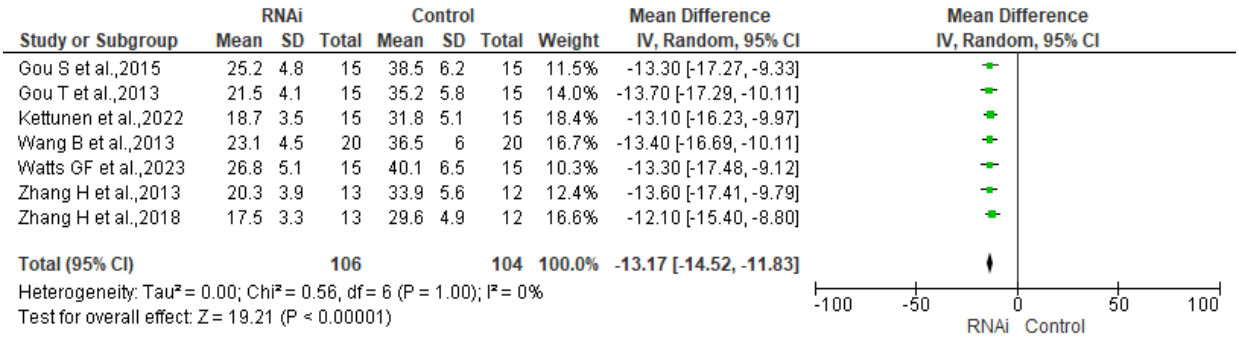

Figure 3. Forest plot of RNAi on lipid content in plaque.

Figure 4 presents a forest plot illustrating the results of the meta-analysis on the effect of RNAi on collagen content within atherosclerotic plaques. This visualization allows us to understand the impact of RNAi interventions on plaque stability, as collagen contributes to the structural integrity and strength of the plaque. All squares are positioned to the right of the vertical line (zero effect line), indicating that every study demonstrated an increase in collagen content within the atherosclerotic plaques following RNAi treatment. The mean differences and their CIs suggest a substantial increase in collagen content across the studies. This implies that RNAi interventions consistently lead to a strengthening of the plaque structure. The overall pooled effect estimate (diamond) shows a statistically significant increase in collagen content with RNAi (MD 5.70; 95% CI 4.34 to 7.05). This confirms that, on average, RNAi interventions significantly increase the collagen content of atherosclerotic plaques compared to controls. The I^2 value of 0% indicates no heterogeneity across the studies. This suggests a high degree of consistency in the findings regarding the effect of RNAi on increasing collagen content in plaques.

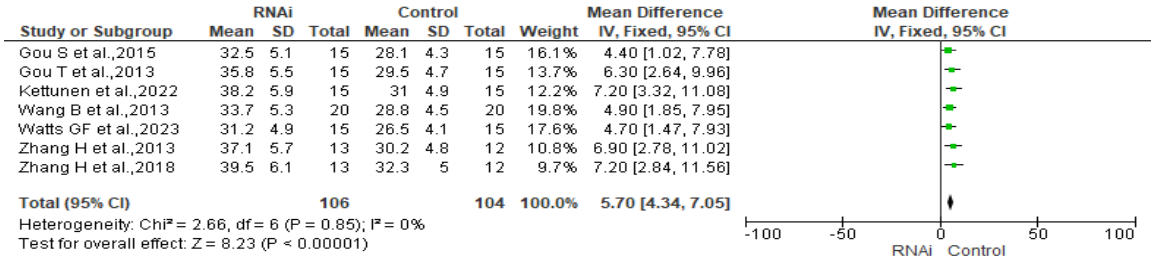

Figure 4. Forest plot of RNAi on collagen content in plaque.

Figure 5 presents a forest plot illustrating the results of the meta-analysis on the effect of RNAi on TNF-alpha levels. TNF-alpha is a key inflammatory cytokine involved in the pathogenesis of atherosclerosis, so understanding the impact of RNAi on its levels is crucial for assessing the therapeutic potential of this approach. All squares are positioned to the left of the vertical line (zero effect line), indicating that every study demonstrated a reduction in TNF-alpha levels following RNAi treatment. The mean differences and their CIs suggest a substantial reduction in TNF-alpha levels across the studies. This implies that RNAi interventions consistently lead to a decrease in inflammation. The overall pooled effect estimate (diamond) shows a statistically significant reduction in TNF-alpha levels with RNAi (MD -11.66; 95% CI -12.50 to -10.82). This confirms that, on average, RNAi interventions significantly decrease TNF-alpha levels compared to controls. The I^2 value of 0% indicates no heterogeneity across the studies. This suggests a high degree of consistency in the findings regarding the effect of RNAi on reducing TNF-alpha levels.

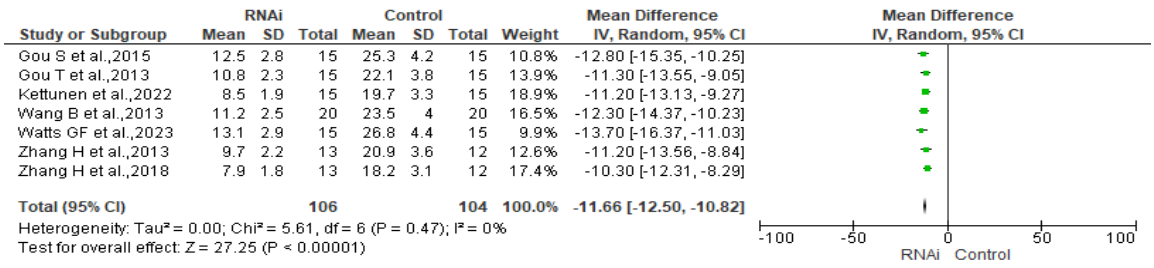

Figure 5. Forest plot of RNAi on TNF alpha.

Figure 6 presents a forest plot illustrating the results of the meta-analysis on the effect of RNAi on IL-6 levels. IL-6 is another key inflammatory cytokine that plays a significant role in the development and progression of atherosclerosis. Analyzing the impact of RNAi on IL-6 levels is important for evaluating its potential to modulate inflammation in the context of this disease. All squares are positioned to the left of the vertical line (zero effect line), indicating that every study demonstrated a reduction in IL-6 levels following RNAi treatment. The mean differences and their CIs suggest a substantial reduction in IL-6 levels across the studies. This implies that RNAi interventions consistently lead to a decrease in inflammation. The overall pooled effect estimate (diamond) shows a statistically significant reduction in IL-6 levels with RNAi (MD -8.67; 95% CI -9.40 to -7.95). This confirms that, on average, RNAi interventions significantly decrease IL-6 levels compared to controls. The I^2 value of 10% indicates low heterogeneity across the studies. This suggests a high degree of consistency in the findings regarding the effect of RNAi on reducing IL-6 levels.

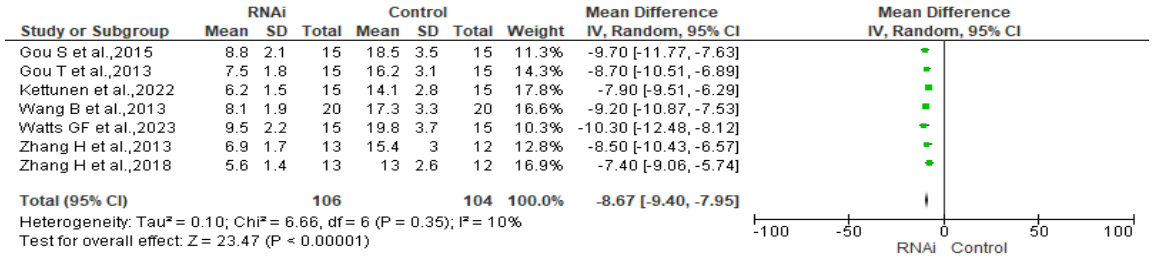

Figure 6. Forest plot of RNAi on IL-6.

Figure 7 provides a forest plot depicting the results of the meta-analysis concerning the effect of RNAi on total cholesterol levels. Given the crucial role of cholesterol in the development of atherosclerosis, understanding how RNAi interventions influence cholesterol levels is vital for assessing their therapeutic potential. All squares are positioned to the left of the vertical line (zero effect line), indicating that every study demonstrated a reduction in total cholesterol levels following RNAi treatment. The mean differences and their CIs suggest a substantial reduction in total cholesterol across the studies. This implies that RNAi interventions consistently lead to lower cholesterol levels. The overall pooled effect estimate (diamond) shows a statistically significant reduction in total cholesterol with RNAi (MD -55.63; 95% CI -65.02 to -46.25). This confirms that, on average, RNAi interventions significantly decrease total cholesterol levels compared to controls. The I^2 value of 0% indicates no heterogeneity across the studies. This suggests a high degree of consistency in the findings regarding the effect of RNAi on reducing total cholesterol.

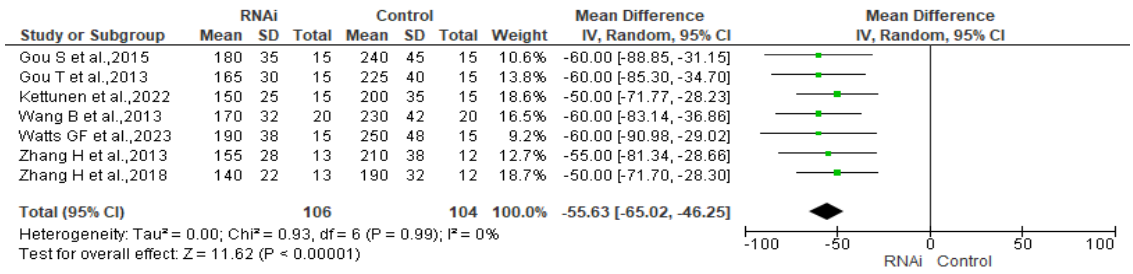

Figure 7. Forest plot of RNAi on total cholesterol.

Figure 8 presents a forest plot illustrating the results of the meta-analysis on the effect of RNAi on LDL-cholesterol levels. As LDL-cholesterol is a major contributor to atherosclerotic plaque formation, understanding the impact of RNAi on its levels is crucial for evaluating its potential to prevent and treat cardiovascular disease. All squares are positioned to the left of the vertical line (zero effect line), indicating that every study demonstrated a reduction in LDL-cholesterol levels following RNAi treatment. The mean differences and their CIs suggest a substantial reduction in LDL-cholesterol levels across the studies. This implies that RNAi interventions consistently lead to lower LDL-cholesterol. The overall pooled effect estimate (diamond) shows a statistically significant reduction in LDL-cholesterol with RNAi (MD -34.04; 95% CI -40.65 to -27.42). This confirms that, on average, RNAi interventions significantly decrease LDL-cholesterol levels compared to controls. The I^2 value of 0% indicates no heterogeneity across the studies. This suggests a high degree of consistency in the findings regarding the effect of RNAi on reducing LDL-cholesterol.

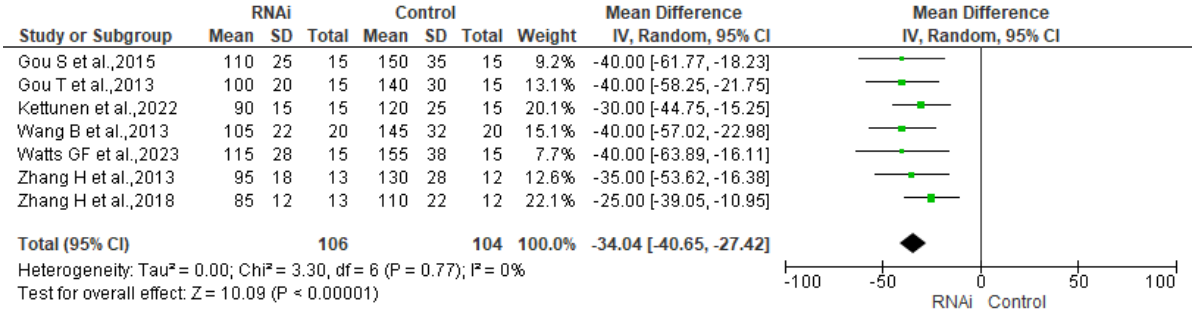

Figure 8. Forest plot of RNAi on LDL-cholesterol.

4. Discussion

Our meta-analysis unequivocally demonstrates that RNAi interventions exert a profound impact on atherosclerotic plaque size and composition in preclinical models. This observation aligns with a growing body of evidence that highlights the potential of RNAi to target various molecular pathways intricately involved in the complex process of atherogenesis. The significant reduction in atherosclerotic plaque size observed in our analysis is a critical finding with far-reaching implications. Atherosclerosis is characterized by the gradual accumulation of lipids, inflammatory cells, and fibrous tissue within the arterial wall, leading to the formation of plaques that progressively obstruct blood flow. This obstruction can lead to ischemia, a condition where tissues are deprived of adequate oxygen and nutrients, potentially resulting in severe clinical consequences like myocardial infarction (heart attack) and stroke. By effectively reducing plaque size, RNAi interventions directly address the core pathology of atherosclerosis. This reduction in plaque burden can potentially restore normal blood flow, alleviate ischemia, and ultimately reduce the risk of adverse cardiovascular events. The ability of RNAi to achieve this effect stems from its capacity to silence genes that drive the key processes involved in plaque formation and growth. Several molecular mechanisms contribute to the observed reduction in plaque size following RNAi interventions. RNAi can target genes involved in lipid uptake, synthesis, and efflux, thereby reducing the accumulation of cholesterol and other lipids within the arterial wall. For instance, silencing genes like PCSK9, which promotes the degradation of LDL receptors, can enhance LDL clearance from circulation and reduce lipid deposition in the vessel wall. Similarly, targeting genes involved in cholesterol synthesis or intracellular lipid transport can further

limit lipid accumulation within the plaque. Chronic inflammation is a hallmark of atherosclerosis, contributing to all stages of the disease process. RNAi can effectively target inflammatory mediators, such as cytokines (TNF-α, IL-6), chemokines (MCP-1), and adhesion molecules (VCAM-1, ICAM-1), thereby dampening the inflammatory response and reducing the recruitment of immune cells to the plaque site. This suppression of inflammation can limit the progression of atherosclerosis and promote a more stable plaque environment. Vascular remodeling, involving the proliferation and migration of smooth muscle cells and the deposition of extracellular matrix components, plays a crucial role in plaque development and stability. RNAi can target genes involved in these processes, such as matrix metalloproteinases (MMPs) and growth factors, to modulate vascular remodeling and promote a more stable plaque phenotype. Beyond reducing the overall plaque burden, RNAi interventions also exert a profound impact on plaque composition. Our analysis revealed a significant decrease in lipid content and a concomitant increase in collagen content within the plaques. This shift towards a more stable plaque phenotype is of paramount importance in preventing acute cardiovascular events. Atherosclerotic plaques are not all created equal. Some plaques are relatively stable and may remain asymptomatic for years, while others are vulnerable and prone to rupture, triggering thrombosis and leading to acute coronary syndromes or stroke. The stability of a plaque is largely determined by its composition. Plaques with a large lipid core and a thin fibrous cap are considered vulnerable. The lipid core, composed mainly of cholesterol and other lipids, is prone to inflammation and necrosis, weakening the plaque structure. The thin fibrous cap, composed mainly of collagen and smooth muscle cells, is less resistant to rupture under the pressure of blood flow. Plaques with a smaller lipid core and a thick fibrous cap are considered more stable. The thick fibrous cap provides greater structural integrity, making the plaque less likely to rupture. By reducing lipid content and increasing collagen content, RNAi interventions effectively shift the balance from vulnerable to stable plaques. As discussed earlier, RNAi can reduce lipid accumulation within the plaque by targeting genes involved in lipid metabolism. This reduction in lipid content not only decreases the size of the lipid core but also reduces inflammation and necrosis, contributing to plaque stability. RNAi can promote collagen synthesis by targeting genes that regulate collagen production or degradation. Collagen is the main structural component of the fibrous cap, and its increased deposition strengthens the cap and enhances plaque stability. Smooth muscle cells play a crucial role in plaque stability by synthesizing collagen and other extracellular matrix components. RNAi can modulate smooth muscle cell function and promote their contribution to plaque stability. The ability of RNAi to promote plaque stabilization has significant clinical implications. By reducing the risk of plaque rupture, RNAi interventions could potentially prevent acute cardiovascular events and improve long-term outcomes for patients with atherosclerosis. This is particularly important for patients with vulnerable plaques, who are at high risk of experiencing life-threatening complications.[11-13]

Our meta-analysis underscores the remarkable ability of RNAi interventions to not only modify plaque size and composition but also to exert a profound influence on two key drivers of atherosclerosis: lipid metabolism and inflammation. By simultaneously targeting these intertwined processes, RNAi offers a promising avenue for a more comprehensive and effective therapeutic strategy against this complex disease. The significant reduction in total cholesterol and LDL-cholesterol levels observed in our analysis highlights the potential of RNAi to precisely modulate lipid metabolism, a central player in the pathogenesis of atherosclerosis. While lowering LDL-cholesterol ("bad" cholesterol) has long been a cornerstone of atherosclerosis treatment, RNAi expands the therapeutic repertoire by offering the ability to target specific genes involved in cholesterol synthesis, uptake, and clearance. A prime example of RNAi's

precision targeting in lipid metabolism is the silencing of proprotein convertase subtilisin/kexin type 9 (PCSK9). PCSK9 is a protein that binds to LDL receptors on the surface of liver cells, promoting their degradation and thereby reducing the liver's capacity to clear LDL-cholesterol from the bloodstream. By silencing PCSK9, RNAi interventions can increase the number of LDL receptors available for LDL uptake, leading to enhanced LDL clearance and lower circulating cholesterol levels. This approach has shown promising results in preclinical studies and is currently being investigated in clinical trials for the treatment of hypercholesterolemia and cardiovascular disease. While PCSK9 silencing represents a significant advancement in lipid-lowering therapy, RNAi's potential extends far beyond this single target. Numerous other genes involved in lipid metabolism can be targeted to achieve a more comprehensive and tailored approach to managing cholesterol levels. HMG-CoA Reductase enzyme is the rate-limiting step in cholesterol synthesis. Silencing HMG-CoA reductase can effectively reduce cholesterol production, complementing the action of statins, which inhibit the same enzyme. Apolipoprotein B (ApoB) is the primary protein component of LDL particles. Silencing ApoB can reduce the production and secretion of LDL, leading to lower circulating LDL-cholesterol levels. Niemann-Pick C1-Like 1 (NPC1L1) is a protein involved in cholesterol absorption in the intestine. Silencing NPC1L1 can reduce cholesterol absorption from the diet, further contributing to lower cholesterol levels. MicroRNAs (miRNAs) play a crucial role in regulating lipid metabolism. Targeting miRNAs that promote cholesterol synthesis or inhibit cholesterol efflux can further fine-tune lipid homeostasis. Beyond its impact on lipid metabolism, RNAi also demonstrates a remarkable ability to dampen the inflammatory response associated with atherosclerosis. This anti-inflammatory action is crucial, as chronic inflammation is a driving force behind all stages of atherosclerosis, from the initial insult to the endothelium to the eventual rupture of vulnerable plaques. Our meta-analysis revealed a significant reduction in the levels of two key inflammatory cytokines, TNF-α and IL-6, following RNAi interventions. These cytokines are produced by various cells within the atherosclerotic plaque, including macrophages, endothelial cells, and smooth muscle cells, and they contribute to a cascade of inflammatory events that promote plaque progression and instability. Tumor necrosis factor-alpha (TNF-α) is a potent pro-inflammatory cytokine that plays a central role in atherogenesis. It promotes endothelial dysfunction, increases vascular permeability, enhances leukocyte recruitment, and stimulates the production of other inflammatory mediators. By silencing TNF-α, RNAi can effectively interrupt this inflammatory cascade and reduce the overall inflammatory burden within the plaque. Interleukin-6 (IL-6) is another key inflammatory cytokine involved in atherosclerosis. It contributes to endothelial dysfunction, promotes the differentiation of monocytes into macrophages, and stimulates the production of acute-phase reactants. By reducing IL-6 levels, RNAi can further dampen the inflammatory response and limit the progression of atherosclerosis. While TNF-α and IL-6 are central players in atherosclerosis-related inflammation, RNAi's potential extends to targeting a wide range of other inflammatory mediators. Chemokines, such as MCP-1, are responsible for attracting monocytes and other immune cells to the site of inflammation. Silencing chemokines can reduce leukocyte recruitment and limit the inflammatory response within the plaque. Adhesion molecules, such as VCAM-1 and ICAM-1, mediate the adhesion of leukocytes to the endothelium, facilitating their entry into the arterial wall. Silencing adhesion molecules can further reduce leukocyte infiltration and inflammation. RNAi can target key signaling pathways involved in inflammation, such as the NF-κB pathway, to broadly modulate the inflammatory response. By targeting various inflammatory mediators and signaling pathways, RNAi interventions can effectively interrupt the vicious cycle of inflammation that drives atherosclerosis progression. This interruption can

lead to a more stable plaque environment, reducing the risk of plaque rupture and thrombosis. Moreover, the anti-inflammatory effects of RNAi can complement its lipid-lowering actions, providing a synergistic therapeutic approach to atherosclerosis.[14-17]

While conventional therapies for atherosclerosis, such as statins, antiplatelet agents, and lifestyle modifications, have undoubtedly revolutionized cardiovascular care and significantly improved patient outcomes, they are not without limitations. These limitations underscore the need for novel therapeutic strategies, and RNAi, with its unique mechanism of action and ability to precisely target disease-related genes, emerges as a promising contender in the fight against this pervasive disease. Statins are the mainstay of lipid-lowering therapy for atherosclerosis, effectively reducing LDL-cholesterol levels and preventing cardiovascular events. They work by inhibiting HMG-CoA reductase, a key enzyme in cholesterol synthesis, thereby lowering cholesterol production in the liver. Not all patients respond equally well to statins, and some individuals may require higher doses or alternative therapies to achieve optimal cholesterol control. Even with statin therapy, a significant residual risk of cardiovascular events remains, highlighting the need for additional therapeutic strategies to further reduce this risk. While statins have some anti-inflammatory properties, their primary effect is on lipid lowering. They may not be sufficient to address the chronic inflammation that drives atherosclerosis progression. Statins can cause muscle pain, liver enzyme elevation, and, in rare cases, more serious side effects like rhabdomyolysis (muscle breakdown). Antiplatelet agents, such as aspirin and clopidogrel, are another cornerstone of atherosclerosis treatment, particularly in patients with established cardiovascular disease. They work by inhibiting platelet aggregation, thereby reducing the risk of thrombus formation and subsequent events like myocardial infarction and stroke. Antiplatelet agents increase the risk of bleeding, which can be a major concern, especially in patients with a history of bleeding or those undergoing surgery. While antiplatelet agents can prevent thrombotic events, they do not directly address the underlying pathology of atherosclerosis, namely plaque formation and progression. Lifestyle modifications, including diet, exercise, and smoking cessation, are essential for preventing and managing atherosclerosis. These modifications can help to lower cholesterol levels, reduce blood pressure, control weight, and improve overall cardiovascular health. However, lifestyle modifications alone may not be sufficient to prevent or reverse atherosclerosis in all individuals, and adherence to these modifications can be challenging for some patients. In contrast to these conventional therapies, RNAi offers a multifaceted approach to atherosclerosis treatment, with the potential to address multiple aspects of the disease simultaneously. RNAi can simultaneously target various molecular pathways involved in atherosclerosis, providing a more comprehensive therapeutic strategy. This is in contrast to statins, which primarily focus on lipid lowering, or antiplatelet agents, which primarily focus on preventing thrombosis. By silencing genes involved in lipid metabolism, inflammation, and vascular remodeling, RNAi can potentially halt or even reverse the progression of atherosclerosis at multiple levels. RNAi's ability to specifically silence disease-related genes offers a level of precision that is not achievable with current therapies. This precision targeting could lead to improved efficacy and reduced side effects. For instance, RNAi can selectively target genes involved in LDL-cholesterol metabolism without affecting HDL-cholesterol ("good" cholesterol), unlike some statins that may lower both. While systemic delivery of RNAi can potentially affect gene expression in various tissues, local delivery strategies can be employed to concentrate the therapeutic effect at the site of atherosclerotic plaques, minimizing off-target effects. This localized approach can potentially enhance efficacy and reduce the risk of systemic side effects. RNAi therapeutics can be delivered directly to the atherosclerotic plaque via a catheter, allowing for precise targeting and minimizing systemic exposure.

RNAi therapeutics can be incorporated into stents, which are small mesh tubes used to prop open narrowed arteries. This allows for sustained release of the therapeutic agent at the site of the plaque. RNAi therapeutics can be encapsulated in nanoparticles, which are tiny particles that can be designed to specifically target atherosclerotic plaques. While conventional therapies primarily focus on managing risk factors and preventing complications, RNAi has the potential to modify the underlying disease process itself. By targeting genes involved in plaque formation and progression, RNAi can potentially halt or even reverse the growth of atherosclerotic plaques, leading to long-term disease modification. RNAi can be used as a complementary therapy alongside conventional treatments, such as statins and antiplatelet agents. By targeting different pathways, RNAi can potentially enhance the efficacy of existing therapies and provide a more comprehensive approach to atherosclerosis management.[18-20]

5. Conclusion

This meta-analysis provides compelling evidence for the therapeutic potential of RNA interference (RNAi) in preclinical models of atherosclerosis. RNAi interventions consistently demonstrated significant reductions in plaque size, improvements in plaque composition (decreased lipid content, increased collagen content), and favorable modulation of lipid metabolism and inflammation. These findings highlight the multifaceted benefits of RNAi in targeting key processes involved in atherogenesis. While challenges remain in translating these preclinical findings to clinical applications, particularly in optimizing delivery systems and ensuring safety, RNAi holds considerable promise as a novel therapeutic strategy for atherosclerosis. Future research should focus on addressing these challenges and further exploring the clinical potential of RNAi to ultimately improve outcomes for patients with cardiovascular disease.

6. References

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