

Effect of Rutin on the ox-LDL-Induced Vascular Endothelial Cell Injury by Regulating NEXN-AS1/miR-410-3p Pathway

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ABSTRACT

This study aims to investigate the effect of rutin on ox-LDL-induced vascular endothelial cell injury, and to analyze whether the mechanism is related to the regulation of NEXN-AS1 and miR-410-3p expression. The CCK-8 method was used to detect the toxicity of rutin of different concentrations (0, 25, 50, 100, 200, 400 $\mu\text{mol/L}$) on human umbilical vein endothelial cells (HUVECs). HUVECs were treated with 100 $\mu\text{g/L}$ ox-LDL for 24 h for establishing HUVECs injury model, and then treated with rutin in different doses. The cell activity and apoptosis rate of HUVECs were analyzed by CCK-8 method and flow cytometry, respectively. The levels of intracellular catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) were measured using commercial kit, and expression levels of NEXN-AS1 and miR-410-3p were detected using real-time quantitative PCR analysis. The dual luciferase experiment was applied to verify the targeted relationship between NEXN-AS1 and miR-410-3p. HUVECs were transfected with NEXN-AS1 overexpression vector, and then treated with ox-LDL for 24 h before detecting the activity, apoptosis rate and oxidative stress level of HUVECs. The low concentrations (25, 50, 100 $\mu\text{mol/L}$) of rutin had no toxic effect on HUVECs, and the activity of HUVECs was significantly reduced after intervention with high concentrations (200, 400 $\mu\text{mol/L}$) of rutin. ox-LDL treatment significantly reduced HUVECs activity, CAT and SOD levels, NEXN-AS1 expression ($P < 0.05$), and increased apoptosis rate, MDA level, and miR-410-3p expression ($P < 0.05$). Rutin (25, 50, 100 $\mu\text{mol/L}$) treatment reduced ox-LDL-induced apoptosis and MDA levels ($P < 0.05$), increased cell viability, CAT and SOD levels ($P < 0.05$), and up-regulated NEXN-AS1 Expression ($P < 0.05$), down-regulated miR-410-3p expression ($P < 0.05$). miR-410-3p is the target gene of NEXN-AS1. Overexpression of NEXN-AS1 could reduce ox-LDL-induced apoptosis and MDA levels ($P < 0.05$), increase cell viability, CAT and SOD levels ($P < 0.05$), and down-regulate miR-410-3p expression ($P < 0.05$). Interference with NEXN-AS1 could reverse the effects of rutin on ox-LDL-induced proliferation, apoptosis and oxidative stress of vascular endothelial cells. It is concluded that rutin had a protective effect on ox-LDL-induced vascular endothelial apoptosis and oxidative injury, and its mechanism may be related to the up-regulation of NEXN-AS1/miR-410-3p pathway.

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Authors' Contribution

FX and GL collected the samples. FX and WF analyzed the data. WF and GL conducted the experiments and analyzed the results. All authors discussed the results and wrote the manuscript.

Key words

Rutin, Vascular endothelial cells, NEXN-AS1, miR-410-3p, Oxidized low-density lipoprotein, Apoptosis, Oxidative stress.

INTRODUCTION

Atherosclerosis (AS) is a progressive arterial inflammatory disease, which is related to the progression of cerebral infarction, peripheral vascular diseases, stroke and other cardiovascular and cerebrovascular diseases. Studies have shown that endothelial injury caused by oxidized low density lipoprotein (ox-LDL) is the initial event and promoting factor of AS, which can destroy the redox balance of vascular endothelial cells and induce apoptosis and injury of endothelial cells (Yan *et al.*, 2019). Therefore, prevention of endothelial injury has become an important strategy to reverse AS. Rutin is a common polyphenol bioflavonoid,

widely existing in many foods and traditional medicines. It has antioxidant, anti-inflammatory, antiallergic, anticancer, antibacterial and antiviral effects and cytoprotective activity (Lin, 2009). Studies have shown that rutin can improve the sensory and motor performance, recognition and memory of ischemia-reperfusion rats, reduce infarct size and relieve neuron loss (Liu *et al.*, 2018). Rutin can also alleviate nerve degeneration and learning and memory disorder caused by anesthesia by inhibiting neuronal apoptosis (Man *et al.*, 2015). However, it is still unknown whether rutin has beneficial effects on ox-LDL-induced vascular endothelial cell injury. Long-chain non-coding RNA (lncRNA) is an RNA molecule with a length of more than 200 nucleotides. It is involved in many cellular processes such as AS cell proliferation, autophagy, aging and apoptosis. Its expression changes are related to vascular cell function, oxidative stress and vascular inflammation. Thus, it is a potential therapeutic target for AS (Bian *et*

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al., 2020; Fasolo *et al.*, 2019). The lncRNA NEXN-AS1 is located at 1p31.1. Studies indicate that the expression of NEXN-AS1 is decreased in human atherosclerotic plaque, and overexpression of NEXN-AS1 can down-regulate the expression of adhesion molecules and inflammatory factors in endothelial cells and inhibit the progression of AS by inhibiting the oligomerization of toll-like receptor 4 (TLR4) and the activity of nuclear factor (NF)- κ B (Hu *et al.*, 2019). The forecast of target gene shows that miR-410-3p is a potential target gene of NEXN-AS1. It is pointed out that lncRNA FTX can induce cell proliferation, inhibit apoptosis and alleviate myocardial cell injury caused by hypoxia-reoxygenation by downregulating miR-410-3p (Li *et al.*, 2020). However, it is not clear whether NEXN-AS1 targets miR-410-3p and participates in ox-LDL-induced vascular endothelial cell injury. Therefore, the purpose of this study was to explore the protective effect of rutin on ox-LDL-induced vascular endothelial cell injury, and to explore its molecular mechanism through NEXN-AS1/miR-410-3p pathway.

MATERIALS AND METHODS

Experimental materials

HUVECs were purchased from China Type Culture Collection Center. ox-LDL was purchased from Peking Union-Biology Co. Ltd. RPMI-1640 culture medium, fetal bovine serum and streptomycin were purchased from Wuhan Procell Biological Company. Rutin (purity greater than 95%, batch number 20181102) was purchased from Beijing Coolaber Technology Co., Ltd. Over-expression plasmid (pcDNA-NEXN-AS1), small interfering RNA (si-NEXN-AS1), empty vector plasmid (pcDNA), experimental primers and recombinant luciferase reporter gene vector were purchased from Shanghai Sangong Biological Company. Cell counting kit (CCK-8), general SYBR qPCR Master Mix, Annexin-V-FITC apoptosis detection kit were purchased from Nanjing Nuoweizhan Biological Company. Rabbit Bcl-2, Bax and GAPDH monoclonal antibodies, sheep anti-rabbit IgG and Ankaang can be purchased from Nanjing Vazyme Biological Company. M-MLV reverse transcriptase and miRNA reverse transcription kit were purchased from Beijing Biolab Biological Company. Malondialdehyde (MDA) detection kit, catalase (CAT) activity detection kit and superoxide dismutase (SOD) activity detection kit were purchased from Beijing Solarbio Biological Company.

Cells, transfection and grouping

HUVECs were inoculated in RPMI-1640 culture medium containing 1% streptomycin double antibody and 10% fetal bovine serum, and cultured in a wet incubator

at 37°C and 5%CO₂. The fluid was changed every 3 days. When the cell fusion degree was 80%, the passage was 1:3. The experiment was carried out on the third generation of logarithmic HUVECs, and the cell injury model was established with 100 mg/l ox-LDL-induced for 24 h (Zhu *et al.*, 2018), which was designated as ox-LDL group. The normal cultured HUVECs were designated as control group (con). To test the effect of rutin on ox-LDL-induced HUVECs, HUVECs were incubated with different concentrations (25, 50, 100 μ mol/L) of rutin in combination with 100 mg/L of ox-LDL for 24h. The HUVECs were marked as ox-LDL+rutin-low dose (L) group and ox-LDL+rutin-high dose group (H) (Yu and Zhou, 2013).

To verify that rutin can protect ox-LDL-induced endothelial cell injury by regulating NEXN-AS1/miR-410-3p pathway, pcDNA-NEXN-AS1, pcDNA, si-NEXN-AS1 were transfected into HUVECs according to Lipofectamine 2000 instructions, and the cells were harvested after transfection for 48h. pcDNA-transfected cells and pcDNA-NEXN-AS1-transfected cells were divided into ox-LDL+pcDNA group and ox-LDL+pcDNA-NEXN-AS1 group with 100 mg/l ox-LDL inducing for 24h. Transfected si-NEXN-AS1 cells were incubated for 24h with a culture medium containing 100 μ mol/L rutin and 100 mg/L ox-LDL, and were classified as ox-LDL+rutin +si-NEXN-AS1 group.

CCK-8 assay for cell viability

To test the toxic effect of rutin on HUVECs, 5×10^3 HUVECs were inoculated into each well of 96-well plate and cultured overnight. Rutin with different concentrations (0, 25, 50, 100, 200, 400 μ mol/L) was used to incubate the cells. One plate of cells was taken out at 24 h and 48 h, respectively, and 10 μ L CCK-8 solution was added to each well to test the effect of rutin on ox-LDL-treated HUVECs. 5×10^3 untransfected or transfected HUVECs were inoculated into each well of 96-well plate and cultured overnight. Rutin and/or ox-LDL were added to incubate the cells for 24 h according to the experimental grouping, and then the cell proliferation activity was tested according to the above steps.

Detection of apoptosis by flow cytometry

The density of HUVECs was adjusted to 3×10^6 cells/mL with 1 $\times$ binding buffer. 5 μ L Annexin V-FITC and 5 μ L PI solution were added into 300 μ L cell suspension, and staining was carried out in black box for 20min. The apoptotic cells were analyzed by flow cytometry.

Detection of Bax and Bcl-2 protein expression by Western blot method

The protein was extracted from HUVECs with RIPA buffer, and quantified by BCA kit. The voltage

was set at 100V, and SDS-PAGE electrophoresis was performed for 100 min. The current was set to 20 mA, and the membranetransfer was carried out overnight with a wet membranetransfer instrument. The polyvinylidene fluoride membrane was sealed with 5% skimmed milk at room temperature for 1 h. After combining with primary antibody at room temperature for 2 h, it was then incubated with goat anti-rabbit at room temperature for 1 h. The membranewas incubated with chemiluminescent reagent for 2 min, then fixed in the membraneholder, developed and fixed immediately. The gray value of the target band was analyzed by Image J software.

Real-time quantitative PCR analysis of the expression of NEXN-AS1 and miR-410-3p

Total RNA was extracted from HUVECs by Trizol reagent. cDNA of NEXN-AS1 was synthesized by M-MLV reverse transcriptase, and cDNA of miR-410-3p was synthesized by miRNA reverse transcription kit. RT-qPCR was performed by SYBR qPCR Master Mix. The relative expression of NEXN-AS1 and miR-410-3p was calculated by $2^{-\Delta\Delta C_t}$ formula. The upstream primer of miR-410-3p was 5'-CGCGAATATAACACAGATGGCCTGT-3', and the downstream primer was 5'-CAGTGCCTGTCGTGGAGT-3; the upstream primer of internal reference U6 was 5'-TGCGGGTGCTCGCTTCGGCAGC-3'; the downstream primer was 5'-CCAGTGCAGGGTCCGAGGT-3'; the upstream primer of NEXN-AS1 was 5'-AAGGAATGAGGCTGAAATGG-3', and the downstream primer was 5'-AGGAAAACCTTGGCCAAAGGT-3'; the upstream primer of internal reference GAPDH was 5'-GGAGCGAGATCCCTCCAAAAT-3', and the downstream primer was 5'-GGCTGTTGTCTACTTCTCATGG-3'.

Detection of MDA, CAT and SOD levels in cells

HUVECs were collected into a centrifuge tube. After centrifugation, the supernatant was discarded. After adding extractive solution, the cells were crushed with 200W ultrasound (ultrasound of 3 s, interval of 10 s, repeating for 30 times). After centrifugation of 8000×g for 10 min at 4°C, the supernatant was placed on ice. The MDA content, CAT and SOD activities in HUVECs were measured according to the kit procedures.

Dual-luciferase report experiment

DIANA Tools predicted that NEXN-AS1 and miR-410-3p had specific complementary binding sequences. Wild-type and mutant sequences of NEXN-AS1 (miR-410-3p and NEXN-AS1 binding sites have been mutated) were cloned into double luciferase reporter vector to construct recombinant reporter plasmids WT-NEXN-AS1 and MUT-NEXN-AS1. The recombinant plasmidswere co-transfected with miR-410-3p mimics and miR-NC into HUVECs, respectively, and the relative luciferase activity of each group of cells after transfection for 48 h was analyzed by dual-luciferase reporter gene detection kit.

Statistical methods

The experimental data were expressed by $\bar{x} \pm s$ of three independent repeated experiments, and were statistically analyzed by SPSS 23.0. The comparison between two groups was made by independent sample *t* test, and the comparison between multiple groups was made by one-way ANOVA and SNK-q test. $P < 0.05$ indicated statistically significant difference.

RESULTS

Effects of rutin on proliferation of vascular endothelial cells

After incubation for 24 h, 200/400 $\mu\text{mol/l}$ rutin inhibited the activity of HUVECs. After incubation for 48h, 0/25/50/100 $\mu\text{mol/l}$ rutin had no obvious effect on the viability of HUVECs, while 200/400 $\mu\text{mol/l}$ rutin significantly inhibited the viability of HUVECs ($P < 0.05$), indicating that high concentration of rutin had certain cytotoxicity to HUVECs. Therefore, rutin of 25/50/100 $\mu\text{mol/L}$ was selected for the experiment (Table I).

Effects of rutin on proliferation and apoptosis of ox-LDL-induced vascular endothelial cells

Compared with control group, ox-LDL group cell viability, NEXN-AS1 expression and Bcl-2 protein expression decreased significantly ($P < 0.05$), while apoptosis rate, miR-410-3p expression and Bax protein expression increased significantly ($P < 0.05$); compared with ox-LDL group, ox-LDL+rutin-L group, ox-LDL+rutin -M group, ox-LDL+rutin -H group showed significantly higher cell viability, expression of

Table I.- Effects of rutin at different concentrations on HUVECs cell activity ($\bar{x} \pm s$, $n=3$).

Time	Rutin ($\mu\text{mol/L}$)					
	0	25	50	100	200	400
24 h	0.53 $\pm$ 0.04	0.55 $\pm$ 0.02	0.56 $\pm$ 0.03	0.58 $\pm$ 0.03	0.46 $\pm$ 0.03*	0.43 $\pm$ 0.04*
48 h	1.17 $\pm$ 0.05	1.18 $\pm$ 0.07	1.17 $\pm$ 0.06	1.21 $\pm$ 0.05	0.94 $\pm$ 0.05*	0.80 $\pm$ 0.04*

* $P < 0.05$ compared with group 0.

Table II.- Effects of rutin on the activity and apoptosis of ox-LDL-induced HUVECs cells ($\bar{x} \pm s$, n=3).

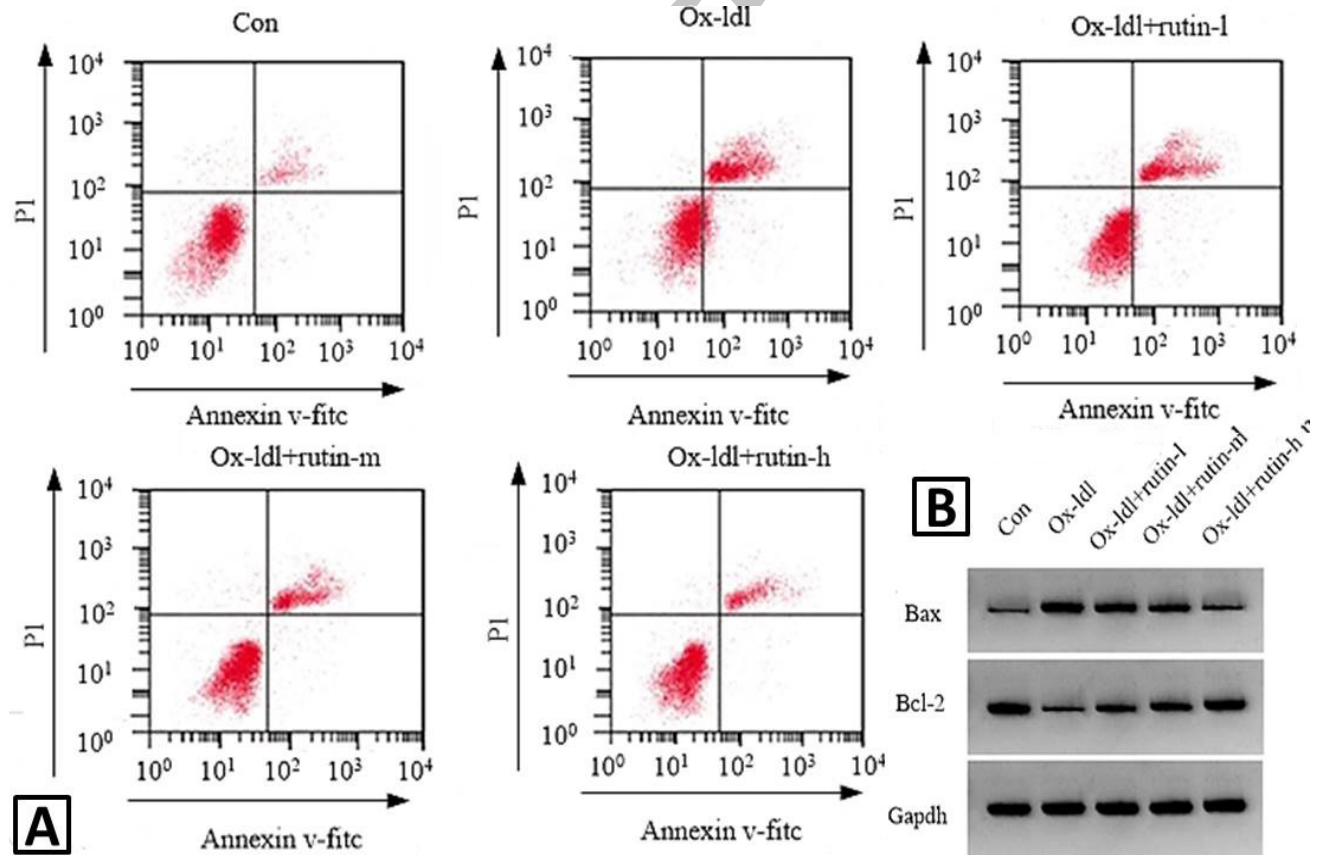
Group	Control	ox-LDL	ox-LDL+rutin -L	ox-LDL+rutin -M	ox-LDL+rutin -H	F	P
NEXN-AS1	1.00±0.00	0.23±0.02*	0.33±0.04 [#]	0.57±0.04 ^{#&}	0.83±0.05 ^{#&@}	260.21	0.000
miR-410-3p	1.00±0.00	4.48±0.15*	3.98±0.11 [#]	2.97±0.09 ^{#&}	1.80±0.06 ^{#&@}	686.27	0.000
OD value	1.23±0.08	0.61±0.03*	0.78±0.04 [#]	0.94±0.06 ^{#&}	1.08±0.05 ^{#&@}	59.37	0.000
Apoptosis rate (%)	6.85±0.49	24.49±0.97*	20.82±0.69 [#]	17.66±0.56 ^{#&}	12.93±0.50 ^{#&@}	320.34	0.000
Bax	0.15±0.01	0.86±0.07	0.64±0.06	0.44±0.04	0.27±0.02	114.72	0.000
Bcl-2	0.89±0.08	0.11±0.01	0.32±0.03	0.53±0.03	0.71±0.06	120.02	0.000

*Compared with control group, $p < 0.05$; [#]compared with ox-LDL group, $p < 0.05$; [&]compared with ox-LDL+rutin -L group, $p < 0.05$; [@]compared with ox-LDL+rutin -M group, $p < 0.05$.

Table III.- Effects of rutin on oxidative stress of ox-LDL-induced HUVECs ($\bar{x} \pm s$, n=3).

Group	Control	ox-LDL	ox-LDL+rutin -L	ox-LDL+rutin -M	ox-LDL+rutin -H	F	P
CAT (U/mL)	62.83±3.52	20.66±0.97*	28.17±1.23 [#]	41.01±1.06 ^{#&}	52.66±2.05 ^{#&@}	221.39	0.000
MDA (nmol/L)	79.13±5.61	181.91±11.70*	154.73±8.97 [#]	126.52±6.68 ^{#&}	102.59±4.15 ^{#&@}	80.08	0.000
SOD (U/mL)	88.25±7.01	20.50±2.65*	37.76±2.56 [#]	57.58±3.50 ^{#&}	73.93±3.83 ^{#&@}	121.95	0.000

*compared with control group, $p < 0.05$; [#]compared with ox-LDL group, $p < 0.05$; [&]compared with ox-LDL+rutin-L group, $p < 0.05$; [@]compared with ox-LDL+rutin -M group, $p < 0.05$.

**Fig. 1.** Effects of rutin on apoptosis of ox-LDL-induced vascular endothelial cells.

NEXN-AS1 and Bcl-2 protein ($P<0.05$), apoptosis rate, miR-410-3p expression, and apoptosis rate. There were significant differences among ox-LDL+rutin-L group, ox-LDL+rutin-M group and ox-LDL+rutin-H group in the above mentioned indexes ($P<0.05$) (Fig. 1; Table II).

Effect of rutin on oxidative stress of ox-LDL-induced vascular endothelial cells

Compared with control group, the levels of CAT and SOD in ox-LDL group cells decreased significantly ($p<0.05$), while the level of MDA increased significantly ($p<0.05$); compared with ox-LDL group, the levels of CAT and SOD in ox-LDL+rutin-L group, ox-LDL+rutin-M group and ox-LDL+rutin-H group increased significantly ($p<0.05$), while the level of MDA decreased significantly ($p<0.05$). There were significant differences among ox-LDL+rutin-L group, ox-LDL+rutin-M group and ox-LDL+rutin-H group ($P<0.05$) (Table III).

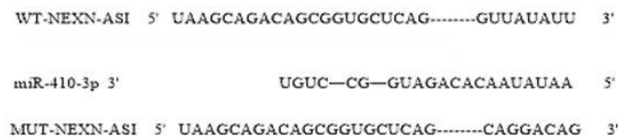


Fig. 2. Complementary sequences of NEXN-AS1 and miR-410-3p.

Targeting relationship between NEXN-AS1 and miR-410-3p

The DIANA Tools prediction results are shown in Figure 2. There are complementary sites between NEXN-AS1 and miR-410-3p sequences. The results of dual-luciferase report experiment are shown in Table IV. The relative luciferase activity of WT-NEXN-AS1 in cells transfected with miR-410-3p mimics was significantly

lower than that transfected with miR-NC ($P<0.05$); compared with miR-NC, the relative luciferase activity of MUT-NEXN-AS1 in cells transfected with miR-410-3p mimics did not change significantly.

Table IV.- Dual-luciferase report experiment ($\bar{x}\pm s$, $n=3$).

Group	WT-NEXN-AS1	MUT-NEXN-AS1
miR-NC	0.96±0.08	1.00±0.09
miR-410-3p	0.28±0.02*	0.97±0.06
t	14.283	0.480
P	0.000	0.656

*compared with miR-NC, $p<0.05$.

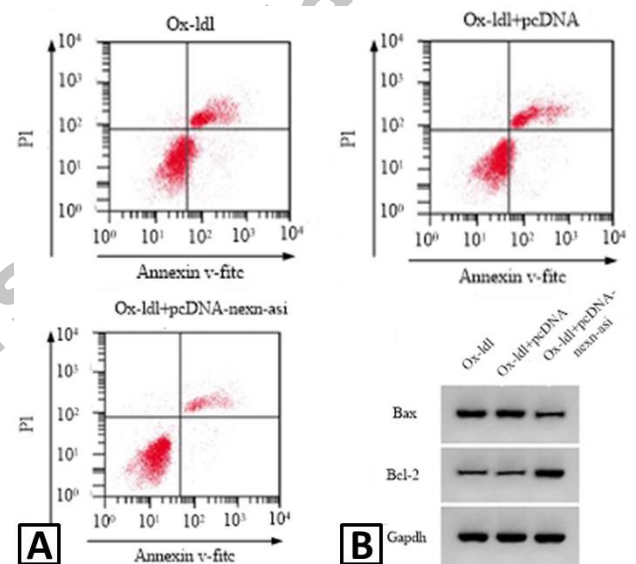


Fig. 3. Effects of overexpression of NEXN-AS1 on apoptosis of ox-LDL-induced vascular endothelial cells.

Table V.- Effects of overexpression of NEXN-AS1 on oxidative stress of proliferation and apoptosis of ox-LDL-induced vascular endothelial cells ($\bar{x}\pm s$, $n=3$).

Group	ox-LDL	ox-LDL+pcDNA	ox-LDL+pcDNA-NEXN-AS1	F	P
NEXN-AS1	0.24±0.02	0.24±0.02	0.90±0.05*#	396.00	0.000
miR-410-3p	4.51±0.19	4.50±0.22	1.45±0.07*#	313.19	0.000
OD value	0.61±0.03	0.58±0.06	1.15±0.09*#	73.50	0.000
Apoptosis rate (%)	24.52±0.93	24.49±0.99	9.35±0.47*#	333.52	0.000
Bax	0.88±0.07	0.85±0.07	0.22±0.02	122.56	0.000
Bcl-2	0.12±0.01	0.13±0.01	0.82±0.08	219.59	0.000
CAT (U/mL)	20.64±1.01	20.75±0.99	57.60±3.12*#	348.19	0.000
MDA (nmol/L)	182.16±11.55	187.28±10.75	89.86±4.28*#	101.22	0.000
SOD (U/mL)	20.63±2.64	20.76±2.54	78.79±5.32*#	242.67	0.000

*compared with ox-LDL group, $p<0.05$; #compared with ox-LDL+pcDNA group, $p<0.05$.

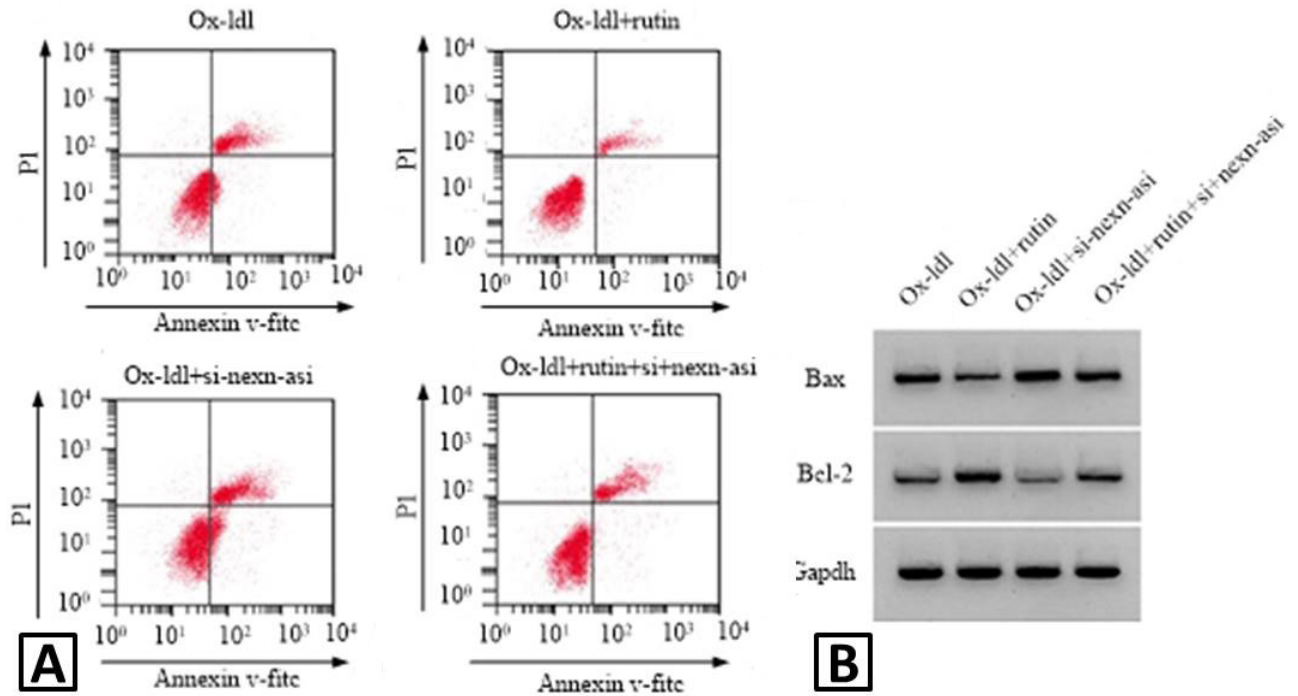


Fig. 4. Interference with NEXN-AS1 could reverse effects of rutin on apoptosis of ox-LDL-induced vascular endothelial cells.

Table VI.- Interference with NEXN-AS1 could reverse effects of rutin on oxidative stress of proliferation and apoptosis of ox-LDL-induced vascular endothelial cells ($\bar{x} \pm s$, $n=3$).

Group	ox-LDL	ox-LDL+rutin	ox-LDL+si-NEXN-AS1	ox-LDL+rutin +si-NEXN-AS1	F	P
NEXN-AS1	0.25±0.02	0.83±0.05*	0.09±0.01*	0.38±0.03 [#]	311.05	0.000
miR-410-3p	4.52±0.15	1.78±0.05*	6.92±0.23*	3.99±0.10 [#]	607.93	0.000
OD value	0.61±0.03	1.10±0.06*	0.52±0.02*	0.74±0.04 [#]	119.92	0.000
Apoptosis rate (%)	24.59±1.07	13.07±0.48*	30.24±1.54*	21.49±0.97 [#]	131.51	0.000
CAT (U/mL)	20.63±1.00	53.01±2.25*	14.35±0.68*	32.18±2.35 [#]	287.84	0.000
MDA (nmol/L)	178.90±5.34	104.32±6.75*	234.59±11.99*	155.76±8.95 [#]	117.57	0.000
SOD (U/mL)	20.66±2.58	74.72±4.26*	11.69±0.57*	35.44±2.35 [#]	303.45	0.000
Bax	0.86±0.07	0.26±0.02	1.19±0.11	0.65±0.04	95.87	0.000
Bcl-2	0.13±0.01	0.73±0.06	0.02±0.01	0.24±0.02	280.19	0.000

*compared with ox-LDL group, $p<0.05$; [#]compared with ox-LDL+rutin group, $p<0.05$.

Effects of overexpression of NEXN-AS1 on proliferation, apoptosis and oxidative stress of ox-LDL-induced vascular endothelial cells

Compared with ox-LDL group and ox-LDL+pcDNA group, the expression of NEXN-AS1, cell viability, Bcl-2 protein, CAT and SOD activities in ox-LDL+pcDNA-NEXN-AS1 group significantly increased, while the expression of miR-410-3p, apoptosis rate, Bax protein and MDA level significantly decreased ($P<0.05$) (Fig. 3; Table V).

Interference with NEXN-AS1 could reverse the effect of rutin on proliferation, apoptosis and oxidative stress of ox-LDL-induced vascular endothelial cells

Compared with ox-LDL group, the expression of NEXN-AS1, cell viability, Bcl-2 protein, CAT and SOD in ox-LDL+rutin group and ox-LDL+si-NEXN-AS1 group significantly increased ($p<0.05$), and the expression of miR-410-3p, apoptosis rate, Bax protein and SOD significantly increased. Compared with ox-LDL+rutin group, the expression of NEXN-AS1, cell vitality, Bcl-2 protein,

CAT and SOD in ox-LDL+rutin +si-NEXN-AS1 group decreased significantly ($P<0.05$), while the expression of miR-410-3p, apoptosis rate, Bax protein and MDA level increased significantly (Fig. 4; Table VI).

DISCUSSION

Rutin, as an effective antioxidant, plays a protective role in the study of various cell injuries. Studies have shown that rutin can improve antioxidant enzyme activity in brain tissue of rats with chronic cerebral hypoperfusion, and improve cognitive dysfunction and brain injury in rats (Qu *et al.*, 2016). Rutin inhibits oxidative stress and apoptosis by up-regulating the expression of 1(SIRT1, thus alleviating myocardial injury induced by hypoxia/reoxygenation (Yang *et al.*, 2019). However, up to now, there are few reports on the effect of rutin on the progression and pathogenesis of AS. In this study, we first studied the toxic effects of different concentrations of rutin on HUVECs, and found that high concentrations of rutin inhibited the viability of HUVECs, suggesting that high concentrations of rutin have cytotoxicity. To study the effect of rutin on the progression of AS, HUVECs model treated with ox-LDL was used to detect the cytoprotective effect of aromatin. The results showed that rutin treatment could inhibit the apoptosis of ox-LDL-induced cells and improve the cell viability. Bcl-2 is an anti-apoptotic protein, while Bax is a pro-apoptotic protein. In this study, rutin treatment could reverse the increase of Bax expression of ox-LDL-induced and prevent the decrease of Bcl-2 expression of ox-LDL-induced, indicating that rutin treatment could significantly reduce the apoptosis of ox-LDL-induced HUVECs. Oxidative stress is the key factor to trigger apoptosis. Intracellular antioxidant enzymes such as CAT and SOD are the first line of defense against the toxic effects of reactive oxygen species and play a key role in the antioxidant defense system (Gao *et al.*, 2019). MDA is the final product of the degradation of polyunsaturated fatty acids by reactive oxygen species, and it is a commonly used marker of oxidative stress (Liu and Liu, 2020). Our study showed that ox-LDL treatment increased MDA content and decreased CAT and SOD levels in HUVECs, while rutin treatment weakened the effect of ox-LDL on these enzymes, indicating that rutin could inhibit oxidative stress induced by ox-LDL. The above studies indicated that rutin could inhibit ox-LDL-induced endothelial cell injury.

In recent years, many studies have confirmed that lncRNA is involved in the regulation of endothelial cell function in the pathogenesis of AS. The research shows that ox-LDL can reduce the level of lncRNA-FA2H-2 in HUVECs, and the down-regulation of lncRNA-FA2H-2 can activate inflammation and inhibit autophagy flux,

thus accelerating AS lesions. Opa interacting protein 5 antisense transcription 1(OIP5-AS1) targets miR-98-5p to regulate TLR4/ nuclear factor κ B(NF- κ B) signaling pathway and accelerate ox-LDL-induced endothelial cell injury. Atorvastatin inhibits the scorch of human vascular endothelial cells by up-regulating the expression of lncRNANEXN-AS1 (Gao *et al.*, 2019). In this study, ox-LDL treatment significantly down-regulated the expression of NEXN-AS1 and up-regulated the expression of miR-410-3p in HUVECs, while rutin treatment significantly reversed the expression changes of ox-LDL-induced NEXN-AS1 and miR-410-3p, suggesting that rutin may protect ox-LDL-induced endothelial cell injury by regulating NEXN-AS1 and miR-410-3p. MiR-410-3p has been confirmed to be related to many human diseases. The level of miR-410-3p in synovium and fibroblast-like cells of patients with rheumatoid arthritis decreases. Knocking down rich transcript 1(NEAT1) can inhibit cell proliferation and promote cell apoptosis by up-regulating miR-410-3p, which is a potential therapeutic target for rheumatoid arthritis (Wu *et al.*, 2020; Wang *et al.*, 2019). In osteosarcoma, magnolin can inhibit the malignant phenotype of tumor cells by up-regulating the expression of miR-410-3p, and increase its sensitivity to cisplatin (Wang *et al.*, 2020). Further studies have confirmed that miR-410-3p is the target gene of NEXN-AS1, and overexpression of NEXN-AS1 obviously weakens the promotion of ox-LDL on miR-410-3p expression, suggesting that there is a regulatory pathway of NEXN-AS1/ miR-410-3p in ox-LDL-induced HUVECs. The functional analysis shows that overexpression of NEXN-AS1 significantly weakens the effects of ox-LDL treatment on HUVECs' activity, apoptosis rate, Bcl-2 and Bax protein expression, MDA level, CAT and SOD activity, and protects HUVECs from ox-LDL-induced apoptosis and oxidative stress, which is similar to the protective effect of rutin on HUVECs. The response experiment shows that interference with the expression of NEXN-AS1 significantly weakens the effects of rutin on the proliferation, apoptosis and oxidative stress of ox-LDL-induced HUVECs. This indicates that the protective effect of rutin on ox-LDL-induced HUVECs may be realized by up-regulating NEXN-AS1/miR-410-3p pathway.

In a word, rutin has protective effect on ox-LDL-induced vascular endothelial cell injury by inhibiting oxidative stress and apoptosis, and its mechanism may be related to up-regulation of NEXN-AS1/miR-410-3p pathway. This preliminarily reveals the possible molecular mechanism of rutin's protective effect on endothelial cells, and provides an important basis for developing rutin for prevention and treatment of AS.

Statement of conflict of interest

The authors have declared no conflict of interests.

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