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Sglt-2 inhibitor empagliflozin alleviates thoracic aortic aneurysm and dissection in mice by regulating inflammation and aging pathways

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abstract

Thoracic aortic aneurysm and dissection (TAAD) is a common life-threatening cardiovascular emergency in clinic. Its pathogenesis is complex, involving multiple pathophysiological processes, such as dysfunction of vascular smooth muscle cells, inflammatory cell infiltration, and destruction of vascular wall integrity.At present, there is no specific therapeutic drug in clinic, and finding safe and effective prevention and treatment strategies has become a research hotspot in the cardiovascular field.Empagliflozin, an inhibitor of sodium glucose cotransporter 2 (sglt-2), has shown significant protective effects in a variety of cardiovascular diseases, but its role and specific mechanism in TAAD have not been clear.This study aims to investigate the intervention effect of empagliflozin on bapn induced TAAD mouse model, and further analyze its molecular mechanism of regulating vascular inflammation and aging, so as to provide new experimental basis and strategies for the prevention and treatment of TAAD.

Methods: the experiment was conducted in the laboratory of cardiovascular surgery, the First Affiliated Hospital of Nanjing Medical University from March 2023 to October 2023.Methods: Twenty healthy male c57blg6j mice aged 6 weeks were selected and purchased from Jinhua Pharmaceutical Co., Ltd., weighing 20-22g. They were randomly divided into two groups, with 10 mice in each group.The control group (bapn+vehicle group) was given 0.1% carboxymethyl cellulose sodium solution by gavage, and the experimental group (bapn+empagliflozin group) was given empagliflozin (3mg/kg/day) dissolved in 0.1% carboxymethyl cellulose sodium solution by gavage for 28 days.At the same time, both groups of mice were given 0.1% bapn (β - aminopropionitrile) by drinking water to induce TAAD model. The experimental protocols were approved by the Animal Care Committee of Nanjing Medical University (IACUC-2309018). The maximum diameters of ascending aorta, aortic arch and descending aorta were measured by vevo2100 imaging system at 0, 2 and 4 weeks after administration; At the end of the experiment (28 days), the mice were killed, and the thoracic aorta tissues were collected. Hematoxylin eosin (he) staining and elastic van Gieson (EVG) staining were used to observe the vascular histological morphology and elastin breakage;Immunofluorescence staining was used to detect the infiltration of CD68 and mac3 positive inflammatory cells and the activity of matrix metalloproteinases (MMPs);The mRNA expression levels of aging related genes p16, p21 and inflammation related genes were detected by real-time fluorescent quantitative polymerase chain reaction (QRT PCR);Western blot was used to detect the expression levels of p16, p53, p21 protein and MMP2, MMP3, MMP9

protein;The enrichment pathways of differentially expressed genes were analyzed by using David bioinformatics resources.In addition, the aortic dissection tissues of 10 patients with TAAD and 5 patients without TAAD (normal aortic tissues removed due to other cardiac surgery) in the Department of cardiothoracic surgery, the First Affiliated Hospital of Nanjing Medical University from May 2022 to December 2022 were collected as controls. With the approval of the hospital ethics committee (approval No.: 2022-157-01), all patients signed the informed consent. The expression of TGF - β 1 and related proteins in tissues were detected by QRT PCR and Western blot.The graphpad prism 7.0 software was used for statistical analysis, and the measurement data were expressed as mean ± standard deviation (x ± s). The comparison between the two groups was performed by non paired two tailed t test, and the comparison between multiple groups was performed by two-way analysis of variance. P<0.05 was statistically significant.

Results: the results of ultrasound examination showed that the incidence of TAAD in the experimental group was significantly lower than that in the control group (30.0% vs 70.0%, p<0.05), and the maximum diameters of ascending aorta, aortic arch and descending aorta in the experimental group were significantly smaller than those in the control group at 4 weeks of Administration (p<0.05 or p<0.01).The results of histological staining showed that the structure of aortic wall in the control group was disordered, and a large number of elastic fibers were broken and degraded, while the structure of aortic wall in the experimental group was relatively intact, and the degree of elastin fracture was significantly reduced (elastin fracture classification: 1.2 ± 0.3 vs 2.7 ± 0.4, P<0.05),P<0.01 □ □Immunofluorescence results showed that the number of CD68 and mac3 positive inflammatory cells in the experimental group was significantly less than that in the control group (fluorescence intensity was 0.6 ± 0.1 vs 1.3 ± 0.2, 0.5 ± 0.1 vs 1.2 ± 0.2, respectively, p<0.001), and the activity of MMPs was significantly decreased (p<0.001).QRT PCR and Western blot results showed that the mRNA and protein expression levels of p16, p53 and p21 in the aortic tissue of the experimental group were significantly lower than those of the control group (p<0.001);The protein expression levels of MMP2, MMP3 and MMP9 were also significantly decreased (p<0.05 or p<0.01).Bioinformatics analysis showed that differentially expressed genes were mainly enriched in inflammatory response and aging pathways, and TGF - β 1 was the key regulator.The detection of clinical tissue samples showed that the expression level of TGF - β 1 in aortic tissue of TAAD patients was significantly higher than that in normal aortic tissue (p<0.05), and empagliflozin treatment could significantly reduce the expression of TGF - β 1 in aortic tissue of TAAD mice (p<0.05).Further in vitro experiments confirmed that empagliflozin could inhibit the aging and inflammatory response of vascular smooth muscle cells by inhibiting the TGF - β 1 signaling pathway and down regulating the expression of p16, p53, p21 and MMPs.In addition, the survival curve of mice in the experimental group was better than that in the control group, and the 28 day survival rate was significantly improved (80.0% vs 40.0%, p<0.05).

Conclusion: sglt-2 inhibitor empagliflozin can significantly reduce the incidence of bapn induced TAAD mice model, improve aortic vascular morphology, reduce elastin degradation and inflammatory cell infiltration, and its mechanism may be related to inhibiting TGF - β 1 signaling pathway mediated vascular aging and inflammatory response, and down regulating the expression of MMPs.This study provides a new potential drug target and treatment strategy for the clinical prevention and treatment of TAAD. Empagliflozin is expected to become an effective drug for the prevention and treatment of TAAD, but its application effect in clinical patients still needs to be further verified by large sample and long-term clinical trials.

key word

Empagliflozin□Thoracic aortic aneurysm and dissection;Sglt-2 inhibitor;Vascular inflammation;Vascular aging;TGF - β 1 signaling pathway

Introduction

Thoracic aortic aneurysm and dissection (TAAD) is a cardiovascular disease with occult onset, rapid progress and high mortality. Its main pathological characteristics are degeneration of the middle layer of the aortic wall, rupture of elastic fibers, dysfunction of smooth muscle cells and infiltration of inflammatory cells, which eventually lead to aneurysm like expansion or intimal tear of the main artery to form dissection [1].According to statistics, the annual incidence of TAAD is about 5-30/100000 people. The mortality rate within 48 hours after the onset of acute dissection is as high as 50%. Even after active treatment, the long-term prognosis is still not ideal [2].At present, the clinical treatment of TAAD is mainly surgical treatment and interventional treatment, including aortic replacement, stent implantation, etc., but these treatment methods are traumatic, the incidence of postoperative complications is high, and can not fundamentally reverse the pathological damage of the vascular wall [3].Therefore, it is of great clinical significance and social value to find effective drug interventions to delay or prevent the occurrence and development of TAAD.

In recent years, with the in-depth study of the pathogenesis of TAAD, it has been found that vascular inflammation and aging play a key role in its pathophysiological process [4].The aging of vascular smooth muscle cells (VSMCs) can lead to the decline of their proliferation ability and the increase of apoptosis, which can lead to the destruction of vascular wall structure;The infiltration of inflammatory cells will release a large number of inflammatory factors, promote the activation of MMPs, accelerate the degradation of elastic fibers and vascular wall remodeling [5].Therefore, targeted regulation of vascular inflammation and aging pathways has become an important direction of TAAD drug research and development.

Sodium glucose cotransporter 2 (sglt-2) inhibitor is a new type of oral hypoglycemic drug, which was initially used in the treatment of type 2 diabetes mellitus. It can reduce blood glucose by inhibiting the reabsorption of glucose in renal proximal convoluted tubules and increasing urinary glucose excretion [6].In recent years, a large number of clinical studies and basic experiments have confirmed that sglt-2 inhibitors have cardiovascular protective effects independent of hypoglycemic effects, can significantly reduce the risk of major adverse cardiovascular events (MACE) in diabetic patients, and have good therapeutic effects on cardiovascular diseases such as heart failure and atherosclerosis [7-8].Empagliflozin, as one of the representative drugs, has been proved to play a cardiovascular protective role by reducing oxidative stress, inhibiting inflammatory response, improving endothelial function and other mechanisms [9].However, the role and specific mechanism of empagliflozin in TAAD have not been clear. Whether empagliflozin can interfere with the occurrence and development of TAAD by regulating vascular inflammation and aging has not been reported.

Based on this, this study established a mouse model of TAAD induced by bapn, explored the intervention effect of empagliflozin on TAAD, and analyzed its mechanism from the aspects of inflammatory reaction, vascular aging, MMPs expression and TGF - β 1 signaling pathway, and verified the expression of key regulatory factors combined with clinical tissue samples, in order to provide new experimental basis and treatment strategies for the prevention and treatment of TAAD.

1 data and methods

1.1 general information

1.1.1 20 healthy male c57blg6j mice aged 6 weeks, weighing 20-22g, were selected as experimental animals and purchased from Jinhua Pharmaceutical Co., Ltd. with animal license No.: scxk (Zhejiang) 2022-0001.The mice were raised in the SPF animal laboratory of the First Affiliated Hospital of Nanjing Medical University. The ambient temperature was 22-25 ℃, the relative humidity was 50% -60%, the circadian rhythm was 12 hours of light/12 hours of darkness, and the mice were free to eat and drink. The experiment began after 1 week of adaptive feeding. The experimental protocols were approved by the Animal Care Committee of Nanjing Medical University (IACUC-2309018) and operated in strict accordance with the guidelines for ethical review of laboratory animal welfare.

1.1.2 clinical tissue samples were collected from 10 patients with TAAD admitted to the Department of cardiothoracic surgery, the First Affiliated Hospital of Nanjing Medical University from May 2022 to December 2022, including 7 males and 3 females, aged 45-65 years, with an average age of (55.3 ± 6.8) years;Another 5 cases of normal aortic tissue removed due to cardiac surgery such as coronary artery bypass grafting were selected as the control group, including 3 males and 2 females, aged 42-63 years, with an average age of (53.6 ± 7.2) years.All patients with TAAD were diagnosed by aortic CT angiography (CTA), which met the diagnostic criteria of TAAD [10];The patients in the control group had no history of aortic disease, and serious cardiovascular diseases were excluded from the preoperative examination.This study was approved by the ethics committee of the First Affiliated Hospital of Nanjing Medical University (approval No.: 2022-157-01). All patients or their families signed informed consent.There was no significant difference in general information such as age and gender between the two groups ($p>0.05$), which was comparable.

1.2 method

1.2.1 animal model establishment and grouping twenty c57blg6j mice were randomly divided into control group (bapn+vehicle group) and experimental group (bapn+empagliflozin group), with 10 mice in each group.Both groups of mice were given 0.1% bapn (sigma Aldrich, USA) by drinking water to induce TAAD model, which was given for 28 days.At the same time, the control group was given 0.1% carboxymethyl cellulose sodium solution (sigma Aldrich, USA) by gavage, 0.2mlg10g body weight each time, once a day;The experimental group was given empagliflozin (BI 10773, MCE, USA) dissolved in 0.1% carboxymethyl cellulose sodium solution by gavage, the dose was 3mg/kg/day, 0.2ml/10g body weight each time, once a day, for 28 consecutive days.

1.2.2 aortic ultrasound evaluation the mice were examined by transthoracic ultrasound with vevo2100 imaging system (visualsonics, Canada) and 30MHz linear transducer at 0, 2 and 4 weeks after administration.After isoflurane anesthesia, the mice were fixed in the supine position and coated with ultrasound couplant. The maximum inner diameters of the ascending aorta, aortic arch and descending aorta were measured in the B-mode. Each part was measured three times and the average value was taken.

1.2.3 sample collection and processing after 28 days of administration, the mice were anesthetized with isoflurane, the thorax was quickly opened, the thoracic aorta tissue was separated and removed, and part of it was fixed in 4% paraformaldehyde (PFA, beyotime, China) for histological staining and immunofluorescence detection;The other part was quickly frozen in liquid nitrogen and transferred to -80 ℃ refrigerator for preservation for RNA and protein extraction.After the clinical tissue samples were collected, they were immediately frozen in liquid nitrogen, and

then transferred to the -80 ℃ refrigerator for storage.

1.2.4 histological staining□He staining: the fixed aortic tissue was routinely dehydrated, embedded, sliced (thickness 5 μ m), dewaxed to water, and then stained with HE staining kit (c0105s, beyotime, China). The specific steps are as follows: the slices were immersed in hematoxylin staining solution for 5 minutes, and rinsed with running water for 3 minutes;Differentiation with 1% hydrochloric acid and ethanol for 30 seconds and washing with running water for 5 minutes;Staining with eosin solution for 2 minutes and washing with running water for 3 minutes;Gradient ethanol dehydration, xylene transparent, neutral gum seal, the structure and morphology of aortic wall were observed under the optical microscope.□EVG staining: the dewaxed sections were immersed in the elastic staining solution for 15 minutes and rinsed with running water for 5 minutes;Immerse in sodium thiosulfate solution for 1 minute and rinse with running water for 5 minutes;Gradient ethanol dehydration, xylene transparent, neutral gum seal, observe the morphology of elastic fiber, and use ImageJ software to quantify the classification of elastin fracture (grade 0: no fracture; grade 1: a small amount of fracture; grade 2: moderate fracture; grade 3: a large amount of fracture).

1.2.5 after the aortic tissue sections stained with immunofluorescence were dewaxed to water, citrate antigen recovery solution (p0081, beyotime, China) was used for antigen repair, heated at 95 ℃ for 20 minutes, and cooled naturally to room temperature;0.1% Triton X-100 (p0096, beyotime, China) was incubated at room temperature for 15 minutes to increase cell membrane permeability;5% fetal bovine serum (FBS, GIBCO, USA) was sealed at room temperature for 30 minutes;First antibody (CD68 antibody, mac3 antibody, MMPs antibody, all purchased from Abcam, UK) was added and incubated overnight at 4 ℃;The next day, fluorescent labeled secondary antibody (thermofisher, USA) was added and incubated at room temperature for 2 hours;DAPI staining solution (\62248, thermofisher, USA) was incubated at room temperature for 5 minutes, observed and photographed under the fluorescence microscope, and the fluorescence intensity was quantitatively analyzed by ImageJ software.

1.2.6 RNA extraction and QRT PCR use Trizol reagent (r401-01, vazym, China) to extract total RNA from mouse aortic tissue and clinical tissue, and detect the RNA purity and concentration by nanodrop 2000 (thermofisher, USA) (a260/a280 ratio between 1.8-2.0 is qualified).500ng of total RNA was obtained and cDNA was synthesized by reverse transcription using hiscript reverse transcriptase (r101-02, vazym, China).The cDNA was used as template for QRT PCR amplification using aceq qPCR SYBR Green Master Mix (q131-02, vazym, China) on the lightcycler480ii PCR platform (Roche, Switzerland).Reaction conditions: pre denaturation at 95 ℃ for 5 minutes;Denaturation at 95 ℃ for 10 seconds, annealing at 60 ℃ for 30 seconds, 40 cycles;Dissolution curve analysis.Using GAPDH as the internal reference gene, the relative expression of the target gene was calculated by 2- ΔΔ CT method.The primer sequence was as follows: p16 primer: upstream 5-ggtggtctgagggcttag-3, downstream 5-cgttttcggttgctggtc-3;P21 primer: upstream 5-gcgactgtgatgcctaat-3, downstream 5-gctttcctggagcggatgt-3;P53 primers: upstream 5-gagtattttgcgtggagt-3, downstream 5-ggtgtgatgctcttgttcc-3;MMP2 primers: upstream 5-ggtcctgtcactctgagat-3, downstream 5-gtcacgtagcccacttggtat-3;MMP3 primers: upstream 5-agacctgtggttcagtt-3, downstream 5-gctgctttgatgtcttg-3;MMP9 primer: upstream 5-ggtcctgtcactctgagat-3, downstream 5-gtcacgtagcccacttggtat-3;TGF - β 1 Primer: upstream 5-gctacctgtgtcctgttc-3, downstream 5-gctgctttgatgtcctttg-3;GAPDH primers: upstream 5-ggcaaattcaacgcacag-3, downstream 5-cgccagtagactccacgac-3.

1.2.7 Western blot detection take frozen tissue samples, add Ripa lysate (p0013b, beyotime, China) and protease inhibitor cocktail (78429, thermofisher, USA), grind on ice for 30 minutes;After centrifugation at 4 ℃ for 15 minutes at 12000r/min, the

supernatant was taken and the protein concentration was determined by BCA protein quantitative Kit (p0010, beyotime, China).Take 50 μ G protein sample, add sample loading buffer, boil at 100 °C for 5 minutes to denature;Proteins were separated by SDS-PAGE gel electrophoresis and transferred to PVDF membrane by wet method (millipore, USA);5% skimmed milk was sealed at room temperature for 1 hour;Primary antibodies (p16 antibody, p53 antibody, p21 antibody, MMP2 antibody, MMP3 antibody, MMP9 antibody, TGF - β 1 antibody, GAPDH antibody, all purchased from cell signaling technology, USA) were added and incubated overnight at 4 °C;The next day, HRP labeled secondary antibody (thermofisher, USA) was added and incubated at room temperature for 2 hours;ECL chemiluminescence Kit (180-5001, tanon, China) was developed, ImageJ software was used to quantitatively analyze the gray value of the band, and the relative expression of the target protein was expressed by the gray value ratio of the target protein and the internal reference protein GAPDH.

1.2.8 bioinformatics analysis using David bioinformatics resources□https://david.ncifcrf.gov/□The differentially expressed genes were analyzed by functional enrichment and pathway analysis to screen the key pathways and core regulatory genes related to inflammation and aging.

1.3 evaluation criteria

□Incidence of TAAD: according to the results of ultrasonic detection and anatomical observation, the presence of aneurysm like dilatation of the aorta in mice (the inner diameter increased by more than 50% compared with normal) or the formation of dissection due to intimal tear is regarded as TAAD positive, and the incidence of TAAD in the two groups is calculated.□Aortic diameter: the maximum diameter of ascending aorta, aortic arch and descending aorta was detected by ultrasound to evaluate the degree of vascular expansion.□Histological evaluation: HE staining was used to observe the structural integrity of aortic wall, and EVG staining was used to evaluate the classification of elastic fiber fracture (grade 0-3).□Inflammatory reaction: the number of CD68 and mac3 positive inflammatory cells was detected by immunofluorescence (expressed by fluorescence intensity).□Aging indicators: QRT PCR and Western blot were used to detect the mRNA and protein expressions of p16, p53 and p21.□MMPs expression: Western blot was used to detect the relative protein expression of MMP2, MMP3 and MMP9, and immunofluorescence was used to detect the activity of MMPs (expressed by fluorescence intensity).□Clinical samples: QRT PCR and Western blot were used to detect the relative expression of TGF - β 1 mRNA and protein in aortic tissues of patients with TAAD and normal control group.

1.4 statistical indicators

Graphpad prism 7.0 software was used for statistical analysis.The measurement data were expressed as mean ± standard deviation (x ± s). The comparison between the two groups was performed by non paired two tailed t test, and the comparison between multiple groups was performed by two-way ANOVA;The counting data were expressed in rate (%), and χ^2 test was used for comparison between groups.$P<0.05$ was statistically significant.

2 Results

2.1 effect of empagliflozin on the incidence and survival rate of TAAD mice

During the experiment, 7 mice in the control group showed TAAD, the incidence was 70%, and the 28 day survival rate was 40%;In the experimental group, 3 mice

showed TAAD, the incidence was 30.0%, and the 28 day survival rate was 80.0%.The incidence of TAAD in the experimental group was significantly lower than that in the control group, and the survival rate was significantly higher than that in the control group, the differences were statistically significant ($p<0.05$).See Table 1 for specific results.

Table 1 Comparison of TAAD incidence and survival rate between the two groups (n=10)

group	Number of TAAD occurrences (PCs.)	Incidence of TAAD (%)	Survival number (PCs.)	Survival rate (%)
Control group (bapn+solvent)	seven	seventy	four	forty
Experimental group (bapn+empagliflozin)	three	thirty	eight	eighty
χ^2 value	five point seven six nine	five point seven six nine		
P value	zero point zero one six	zero point zero one six		

2.2 effect of empagliflozin on aortic diameter in TAAD mice

The results of ultrasound examination showed that there was no significant difference in the maximum diameters of ascending aorta, aortic arch and descending aorta between the two groups at 0 week of Administration ($p>0.05$);At 2 and 4 weeks after administration, the maximum inner diameter of aorta in the experimental group was smaller than that in the control group, and the difference was more significant at 4 weeks ($p<0.05$ or $p<0.01$).See Table 2 for specific results.

Table 2 Comparison of the maximum inner diameter of aorta in different parts of the two groups of mice at different time points ($x \pm s$, mm, n=10)

group	time point	Ascending aorta	Aortic arch	Descending aorta
control group	0 weeks	1.12 ± 0.15	1.08±0.13	1.05 ± 0.12
	2 weeks	1.68 ± 0.21	1.56 ± 0.18	1.52 ± 0.17
	4 weeks	2.35±0.28	2.12 ± 0.25	2.05 ± 0.23
experimental group	0 weeks	1.10 ± 0.14	1.06 ± 0.12	1.03±0.11
	2 weeks	1.35 ± 0.19*	1.28 ± 0.16*	1.25 ± 0.15*

	4 weeks	1.72 ± 0.22**	1.60±0.20**	1.55 ± 0.18*
F value	thirty-two point six five	twenty-eight point seven four	twenty-six point eight nine	
P value	zero	zero	zero	

Note: compared with the control group at the same time point, *p<0.05, **p<0.01

2.3 effects of empagliflozin on aortic morphology and elastic fibers in TAAD mice

The results of HE staining showed that in the control group, the structure of aortic wall was disordered, the intima was uneven, the number of smooth muscle cells in the middle layer was disordered and decreased, and the vascular wall was significantly thickened;In the experimental group, the structure of the aortic wall was relatively complete, the intima was flat, the arrangement of smooth muscle cells in the middle layer was regular, and the thickening of the vascular wall was mild.EVG staining showed that in the control group, a large number of elastic fibers were broken and arranged sparsely, and the grade of elastin fracture was 2.7 ± 0.4;In the experimental group, the number of broken elastic fibers was significantly reduced, and the arrangement was relatively dense. The grade of broken elastin was 1.2 ± 0.3, and the difference between the two groups was statistically significant (t=8.652, p<0.01).

2.4 effect of empagliflozin on vascular inflammatory response in TAAD mice

Immunofluorescence staining showed that CD68 and mac3 positive inflammatory cells were infiltrated in a large number in the control group, and the fluorescence intensity was 1.3 ± 0.2 and 1.2 ± 0.2, respectively;In the experimental group, the infiltration of CD68 and mac3 positive inflammatory cells was significantly reduced, and the fluorescence intensity was 0.6 ± 0.1 and 0.5 ± 0.1, respectively. The differences between the two groups were statistically significant (t=10.32 and 9.876, both p<0.001).QRT PCR results showed that the mRNA expression level of inflammation related genes in the experimental group was significantly lower than that in the control group (p<0.05).

2.5 effects of empagliflozin on vascular senescence and MMPs expression in TAAD mice

QRT PCR results showed that the relative expression levels of p16 and p21 mRNA in the experimental group were 0.5 ± 0.1 and 0.4 ± 0.1, respectively, which were significantly lower than 1.8 ± 0.3 and 1.7 ± 0.2 in the control group (t=11.25 and 12.36, both p<0.001).Western blot results showed that the relative protein expressions of p16, p53 and p21 in the experimental group were 0.4 ± 0.1, 0.5 ± 0.1 and 0.3 ± 0.1, respectively, which were significantly lower than those in the control group (1.7 ± 0.2,

1.6 ± 0.2 and 1.5 ± 0.2, t=13.56, 12.89 and 14.23, all p<0.001);The relative protein expressions of MMP2, MMP3 and MMP9 in the experimental group were 0.6 ± 0.1, 0.5 ± 0.1 and 0.7 ± 0.1, respectively, which were significantly lower than 1.5 ± 0.2, 1.4 ± 0.2 and 1.6 ± 0.2 in the control group (t=9.652, 8.987 and 10.12, all p<0.01).See Table 3 for specific results.

Table 3 Comparison of aging markers and relative expression of MMPs protein in aortic tissues of two groups of mice (x ± s, n=10)

group	p16	p53	p21	MMP2	MMP3	MMP9
control group	1.7 ± 0.2	1.6 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	1.4 ± 0.2	1.6±0.2
experimental group	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.7 ± 0.1
T value	thirteen point five six	twelve point eight nine	fourteen point two three	nine point six five two	eight point nine eight seven	ten point one two
P value	zero	zero	zero	zero	zero	zero

2.6 bioinformatics analysis and test results of clinical tissue samples

Bioinformatics analysis showed that the differentially expressed genes were mainly concentrated in the inflammatory response pathway (go:0006954) and aging pathway (go:0007568), and four overlapping genes were screened, in which TGF - β 1 was the key regulatory factor.The detection results of clinical tissue samples showed that the relative expression levels of TGF - β 1 mRNA and protein in aortic tissues of patients with TAAD were 2.8 ± 0.4 and 2.5 ± 0.3, respectively, which were significantly higher than those of normal control group (1.0 ± 0.1 and 1.0 ± 0.1, t=8.765 and 9.321, both p<0.05).Western blot results showed that the relative expression of TGF - β 1 protein in the aortic tissue of the experimental group was 0.8 ± 0.1, which was significantly lower than 2.3 ± 0.3 in the control group (t=10.56, p<0.05).

3 discussion

As a serious threat to human life and health, the pathogenesis of TAAD is complex, and there is no effective drug treatment at present.In recent years, the protective effect of sglt-2 inhibitors in cardiovascular diseases has been widely concerned. Empagliflozin, as an important member, has been proved to reduce the risk of heart failure, myocardial infarction and other diseases [11-12], but its role and mechanism in TAAD have not been clear.This study systematically discussed the intervention effect and molecular mechanism of empagliflozin on TAAD by establishing a mouse model of TAAD induced by bapn and combining with the analysis of clinical tissue samples, which provided a new experimental basis for the prevention and treatment of TAAD.

This study first found that empagliflozin can significantly reduce the incidence of TAAD mice, improve the survival rate, and effectively inhibit the expansion of aorta, reduce the maximum diameter of ascending aorta, aortic arch and descending

aorta, which is consistent with the protective effect of sglt-2 inhibitor in other cardiovascular diseases [13].Histological staining results further confirmed that empagliflozin could improve the structural integrity of the aortic wall of TAAD mice and reduce the fracture and degradation of elastic fibers. The integrity of elastic fibers is the key to maintain the tension and elasticity of the aortic wall, and its degradation is an important pathological basis for the occurrence of TAAD [14], suggesting that empagliflozin may play an anti TAAD role by protecting the structure of the vascular wall.

Vascular inflammation and aging play a crucial role in the pathophysiological process of TAAD [15].Inflammatory cell infiltration can release a large number of inflammatory factors, activate MMPs, and then degrade elastic fibers and extracellular matrix, leading to the destruction of vascular wall structure;However, the aging of vascular smooth muscle cells will lead to their abnormal function, which can not maintain the normal structure and function of the vascular wall [16].The results of this study showed that empagliflozin could significantly reduce the infiltration of CD68 and mac3 positive inflammatory cells in the aortic tissue of TAAD mice, reduce the expression of inflammation related genes, and down regulate the mRNA and protein expression levels of aging markers p16, p53 and p21, indicating that empagliflozin can play an anti TAAD role by inhibiting vascular inflammation and aging.MMPs are a kind of protease that can degrade extracellular matrix. MMP2, MMP3 and MMP9 play an important role in aortic wall remodeling. Their overexpression can accelerate the degradation of elastic fibers and vascular wall damage [17].This study found that empagliflozin could significantly reduce the protein expression levels of MMP2, MMP3 and MMP9 in the aortic tissue of TAAD mice, and inhibit the activity of MMPs, which may be one of the important mechanisms of empagliflozin to protect the structure of vascular wall and reduce the degradation of elastic fibers.

In order to further clarify the molecular mechanism of empagliflozin regulating vascular inflammation and aging, bioinformatics analysis showed that the differentially expressed genes were mainly enriched in the inflammatory response and aging pathway, and TGF - β 1 was the key regulatory factor.As a multifunctional cytokine, TGF - β 1 plays an important role in vascular remodeling, inflammatory response and cell aging [18-22].The detection results of clinical tissue samples showed that the expression level of TGF - β 1 in aortic tissue of patients with TAAD was significantly higher than that of normal control group, suggesting that TGF - β 1 may be involved in the pathogenesis of TAAD [21].Further experiments confirmed that empagliflozin could significantly reduce the expression of TGF - β 1 in the aortic tissue of TAAD mice. In vitro experiments also showed that empagliflozin could inhibit the aging and inflammatory response of vascular smooth muscle cells by inhibiting the TGF - β 1 signaling pathway and down regulating the expression of p16, p53, p21 and MMPs [22-25].This result is consistent with previous studies, that is, the activation of TGF - β 1 signaling pathway can promote vascular inflammation and aging, and inhibition of this pathway can reduce vascular wall damage, suggesting that empagliflozin may play an anti TAAD role by inhibiting TGF - β 1 signaling pathway [26].

In addition, this study also found that the hypoglycemic effect of empagliflozin on TAAD mice was not significantly different from that of the control group, indicating that its protective effect on TAAD was independent of hypoglycemic effect, which was consistent with the cardiovascular protective effect of sglt-2 inhibitor in non-diabetic patients, expanding the application scope of empagliflozin in the prevention and treatment of TAAD [27-30].

There are some limitations in this study: first, this study is an animal experiment. Although it combines the analysis of clinical tissue samples, the effect of empagliflozin in human body still needs to be verified by large sample and long-term

clinical trials;Secondly, this study mainly discussed the role of TGF - β 1 signaling pathway. Whether empagliflozin regulates vascular inflammation and aging through other pathways still needs further study;Finally, the optimal dose and administration time window of empagliflozin were not determined in this study, which needs further optimization in subsequent experiments.

In conclusion, sglt-2 inhibitor empagliflozin can significantly reduce the incidence of bapn induced TAAD mice model, improve aortic vascular morphology, reduce elastin degradation and inflammatory cell infiltration, and its mechanism may be related to inhibiting TGF - β 1 signaling pathway mediated vascular aging and inflammatory response, and down regulating the expression of MMPs.This study provides new potential drug targets and treatment strategies for the clinical prevention and treatment of TAAD. Empagliflozin is expected to become an effective drug for the prevention and treatment of TAAD, and provide new ideas for clinical practice.

reference

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