THE QUEST FOR A MEDICAL TREATMENT OF AORTIC STENOSIS: PUTATIVE THERAPEUTIC TARGETS

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ABSTRACT

Aortic stenosis (AS), i.e. calcification and obstruction of the aortic valve (AV), is the most common type of valvular heart disease. The therapeutic options for AS are currently limited to either AV replacement surgery or transcatheter AV implantation. In contrast, no medical treatment has proven effective in slowing the process of valve calcification. The molecular and cellular pathophysiology of AS is an active and complex process, with components of inflammation, lipid accumulation, valvular remodelling, dystrophic calcification, oxidative stress, apoptosis, and heterotopic ossification. These pathways contain several potential targets for medical treatment, which are discussed in the present review. These include the targeting of lipids and lipoproteins, inflammation, and calcification pathways, which have been explored in experimental, epidemiological, and prospective studies. However, further mechanistic studies and prospective trials are needed to better understand the pathophysiology of AS and to lead to new therapeutic strategies for the prevention, or at least the delay, of either surgical or transcatheter valve implantations.

Keywords: Aortic stenosis, echocardiography, inflammation, leukotrienes, valvular heart disease.

INTRODUCTION

Aortic stenosis (AS), i.e. calcification and obstruction of the aortic valve (AV), is the most common type of valvular heart disease. The therapeutic options for AS are currently limited to invasive procedures, such as aortic valve replacement surgery (AVRS) or transcatheter aortic valve implantation (TAVI). In contrast, no medical treatment has proven to be effective in slowing the process of AV calcification.

Based on the original histological description of AV calcification in 1904 by Mönckeberg,[1] calcified AS was for a long time considered as a purely degenerative process associated with aging and being the consequence of calcium deposits on the surface of the valve. Nowadays however, and over the last two decades, the aspect of AS as a chronic inflammatory disease has emerged, and supplanted other theories.[2,3] This inflammatory theory has radically changed the view of AS pathophysiology into an active process of remodelling and valvular calcification, and opened up the quest for a medical treatment of this disease.

Indeed, several key phenomena have been identified as part of the calcification process, with potential therapeutic interest. Examples of such potential medical strategies include targeting lipids and lipoproteins, osteogenic pathways, inflammatory mediators, and proteases. The poor prognosis and increased mortality associated with AS after the onset of symptoms in the absence of either AVRS or TAVI[4] stresses the importance of seeking medical treatments which would slow down the progression of the disease. This article will review the molecular and cellular pathophysiology as well as potential new therapeutic targets of AS.

CELLULAR AND MOLECULAR MECHANISMS OF AS

Based on the histological examinations of the human explanted AV with different degrees of

stenosis, a continuum of pathophysiological changes has been identified,[2] as indicated in Figure 1. Put simply, these processes can be divided into an early initiating stage which is characterised by subendothelial thickening at the aortic side of the valvular leaflets and the presence of lipids; followed by a progression of the disease characterised by inflammation, valvular remodelling, and dystrophic calcification, and finally the end-stage disease with altered valvular structure and heterotopic bone formation (Figure 1).

Early-Stage Disease Processes

The mechanical stress on the AV[5] in combination with hyperlipidaemia and other proatherogenic factors induce endothelial cell activation, which may serve as a starting point for the transition from a normal valve towards a thickened structure (Figure 1). This early stage of AS is also characterised by the recruitment of inflammatory cells, such as macrophages and T cells, which have been detected in valvular lesions.[2,3] Activated leukocytes are a source of proteases, e.g. matrix metalloproteinases (MMP), which induce a degradation of the extracellular matrix (ECM), resulting in valve remodelling and structural alterations as depicted in Figure 1. The resulting thickening and hardening of the AV are, in turn, associated with valvular dysfunction, and as a consequence, further negative alterations of the mechanical stress on the AV.[5] Collectively, this results in a vicious circle of increased inflammation and a narrowing of the AV. Valvular remodelling ranges from simple thickening of the valve to severe calcification, resulting in a significant limitation of the cusp opening. In this process, there may be a potentially vulnerable and reversible disease phase, characterised by increased inflammation which may involve several inflammatory mediators, including transforming growth factor beta (TGF β), interleukin-1 beta (IL-1 β), and tumour necrosis factor-alpha (TNF-α) (Figure 1).

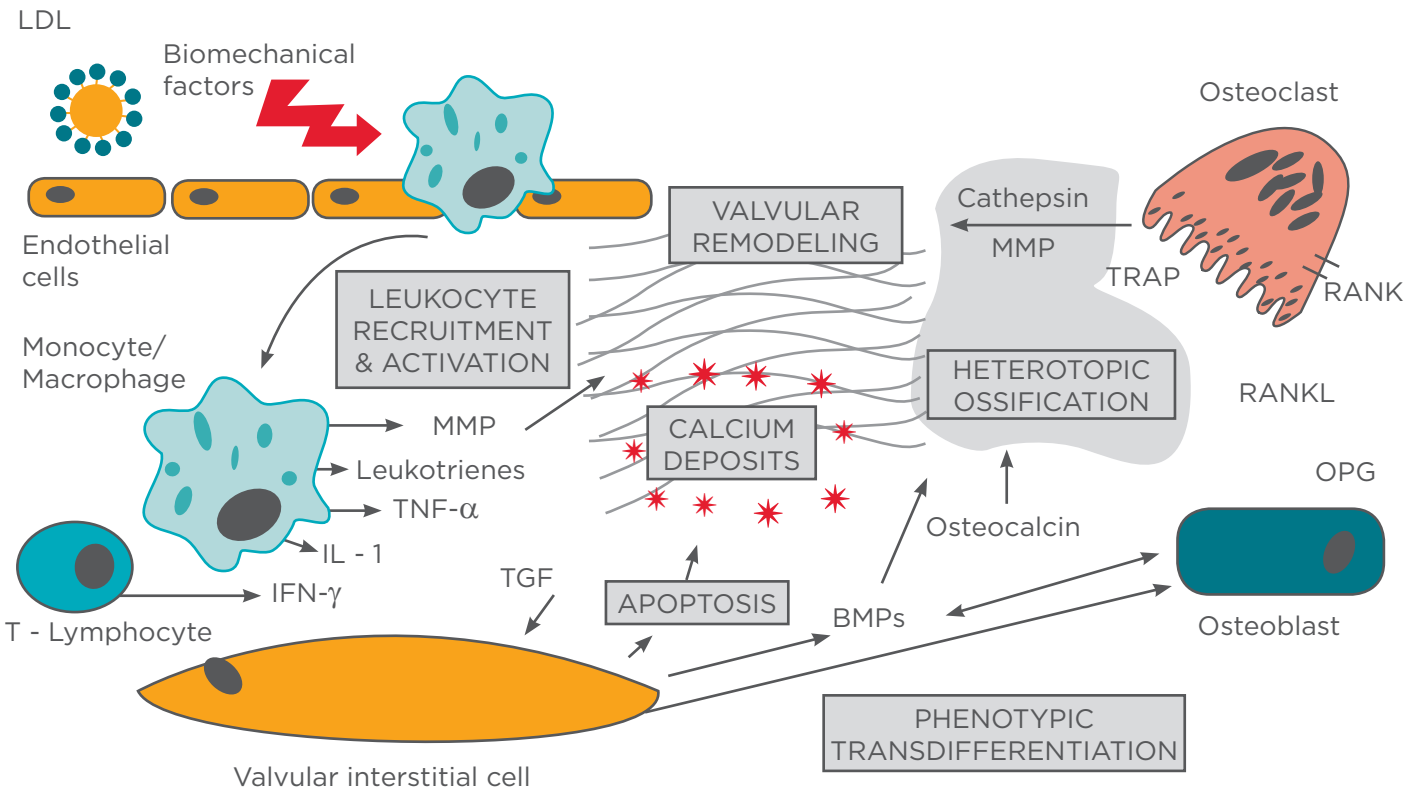

Figure 1: Cellular and molecular mechanisms of aortic stenosis.
The figure shows an overview of some of the early, intermediate, and late processes associated with the development of AS, with examples of cells and mediators involved in valvular remodelling, dystrophic calcification, and heterotopic bone formation within the AV.
AS: aortic stenosis; AV: aortic valve; LDL: low density lipoprotein; MMP: matrix metalloproteinase; TNF: tumour necrosis factor; IL: interleukin; IFN: interferon; BMP: bone morphogenic protein; OPG: osteoprotegerin; RANK: receptor activator of nuclear factor kappa B; RANKL: RANK ligand; TRAP: tartrate-resistant acid phosphatase; TGF β: transforming growth factor beta.

Table 1: Studies of statins in aortic stenosis.

Study	N	Treatment	Follow-up	Baseline Characteristics (Placebo/treated)	AS progression
SALTIRE[16]	155	Atorvastatin 80 mg	2.1 years	Age 68/68 years Female 32/28 years TAV 97/96% V_{max} 3.45/3.39 m/s LDL 3.54/3.45 mol/L	V_{max} ↑ 0.199 m/s/year (placebo) ↑ 0.203 m/s/year (atorvastatin) Calcification ↑ 21.7%/year (placebo) ↑ 22.3%/year (atorvastatin)
SEAS[17]	1,873	Simvastatin 40 mg + Ezetimibe 10 mg	4.4 years	Age 67/68 years Female 39/39% TAV 94/95% V_{max} 3.1/3.1 m/s LDL 3.59/3.62 mmol/L	V_{max} ↑ 0.16 m/s/year (placebo) ↑ 0.15 m/s/year (E+S) P_{mean} ↑ 2.8 mmHg/year (placebo) ↑ 2.7 mmHg/year (E+S)
ASTRONOMER[18]	269	Rosuvastatin 40 mg	3.5 years	Age 58/58 years Female 19/15% TAV 46/55% V_{max} 3.16/3.19 m/s LDL 3.18/3.12 mmol/L	P_{mean} ↑ 6.1 mm Hg/year (placebo) ↑ 6.3 mm Hg/year (rosuvastatin) AVA ↓ 0.08 cm^2/year (placebo) ↓ 0.07cm^2/year (rosuvastatin)

AS: aortic stenosis; Vmax: maximum velocity; LDL: low density lipoprotein; TAV: transcatheter aortic valve; Pmean: mean aortic valve gradient.

Disease Progression

Thickened portions of the AV without macroscopically apparent calcification histologically present spots of mineralisation at this stage, as initially described by Otto et al.[2] and illustrated in Figure 1. The presence of these small calcifications reflects the deposition of hydroxyapatite and other calcium phosphates and is referred to as dystrophic calcification. One of the initiators of dystrophic calcification in the valve is the elastin degradation by gelatinases, and MMP-9 expression and activity is increased in AS.[6,7]

The biomechanical and biochemical environment of the structurally altered AV may have unfavourable effects on the survival of valvular interstitial cells (VIC), and apoptotic VICs have been described as a locus for calcium deposits (Figure 1).

End-Stage Disease Processes

The VICs are at the same time characterised by a high phenotypic plasticity and can undergo transdifferentiation in the AV to an osteoblast phenotype associated with an expression of osteogenic proteins (Figure 1).[8] Although the exact mechanism today remains largely unknown, the evolution towards osteoblasts is facilitated by the transcription factor RUNX2/Cbfa1. In addition, transdifferentiation of VIC may involve epigenetic changes in terms of promoter hypomethylation.[9]

Valvular osteoblasts secrete osteogenic proteins, such as bone morphogenetic proteins (BMP), which signal through the Wnt/β-catenin pathway to induce active osteogenesis, referred to as heterotopic ossification.[10] In addition, osteocalcin, which participates in bone calcification, also represents a factor specific to osteoblasts in AS (Figure 1). Biomechanical stresses can induce microfractures in the heterotopic ossification of the AV.[5] At this time point, osteoclasts are formed by the fusion of mononuclear circulating precursors, and participate in a process of bone remodelling, which may further aggravate the valve calcification, which is discussed below.[11] It should also be mentioned that inflammation is not limited to the early stage of AS, but also appears to remain active in the calcified valve tissue, suggesting that inflammation also plays an active role in heterotopic ossification.

TARGETING LIPIDS AND LIPOPROTEINS

Parallels Between AS and Atherosclerosis

AS pathophysiology shares several of the above-described processes with the arterial calcification encountered in atherosclerosis, such as inflammatory infiltration, lipid accumulation, biomechanical factors, ECM remodelling, and deposition of calcium. In addition, there is a parallel between the epidemiology of AS and atherosclerosis. Among the risk factors for AS (ageing aside) hyperlipidaemia, hypertension, kidney failure, and diabetes can also be included. Nevertheless, in almost half of the cases, patients with AS present no underlying atherosclerotic lesions in their coronary arteries.[12] These findings suggests that, despite morphological and epidemiological similarities, the pathophysiology of AS and that of atherosclerosis are not identical.

Statins in AS

These similarities in terms of atherosclerotic vessel disease and AV stenosis raised the hypothesis that lipid-lowering therapy would retard stenosis progression, similar to its effects on atherosclerosis and its ischaemic complications. This notion received support from the promising effect of statins on the process of calcification *in vitro*.[13] In addition, several observational retrospective studies supported the concept of decreased haemodynamic progression of AS in statin-treated patients.[14,15] In addition, a non-randomised and open-label trial of 121 subjects who had indication for statin treatment (because of elevated low-density lipoprotein [LDL]) showed a decreased echocardiographic progression of AS severity by rosuvastatin treatment.[16] However, prospective studies have not provided support for the treatment of AS with cholesterol lowering drugs. The results of the three major randomised controlled trials (RCTs) evaluating the effects of different lipid lowering therapies on AS progression[17-19] are presented in Table 1. Although there are several differences between the study populations in terms of, for example, age, sex-distribution, and the proportion of bicuspid and tricuspid valves, the subjects shared LDL cholesterol levels below those that may motivate statin treatment as primary prevention (Table 1).

Importantly, the subjects included in the three RCTs listed in Table 1 exhibited similar haemodynamic parameters on echocardiography, with a maximum velocity (V_{max}) over the AV between 3 and 4 m/s, indicative of a moderate AS at the start of the treatment. The follow-up time ranged from 2-4 years, and the yearly progression was ≈0.2 m/s for V_{max} (Table 1). None of the studies showed any significant differences between placebo and treated groups for the echocardiographic parameters of AS progression studied (Table 1). It has been suggested that the failure of these clinical trials was due to a recruitment of patients at a too-late stage of AS. A medical intervention at an earlier stage, in a potentially reversible phase of valvular remodelling, could have been more appropriate than at an established moderate stenosis, which may represent an already non-modifiable phase of the disease. However, a recent *post hoc* analysis of 23,508 participants from three RCTs comparing high (80 mg) and lower doses of atorvastatin did not reveal a dose-dependent effect of atorvastatin treatment on the incidence of clinically diagnosed AS during the median follow-up time of 4.9 years.[20] Even though the last word of the story of statins in AS is probably not yet told, currently the treatment of AS by statins cannot be anticipated in the absence of other indications.

Lipoprotein(a)

In a genome-wide association study of 6,942 participants derived from three different cohorts, a single nucleotide polymorphism in the gene encoding lipoprotein(a) (Lp[a]) was significantly associated with AV calcification (as determined by computed tomography [CT]), as well as with incident AS.[21] Lp(a) is a LDL-like lipoprotein

containing apolipoprotein B-100, which can be linked to a lipoprotein-associated phospholipase (Lp-pLA$_2$), an enzyme which hydrolyses oxidised phospholipids (Figure 2).[22] Both Lp(a) and Lp-PLA$_2$ have previously been identified as risk markers for atherosclerosis, and inhibitors of Lp-PLA$_2$ were developed for the treatment of coronary artery disease (CAD).[22] However, in a recent large multicentre RCT of patients with stable coronary disease, the Lp-PLA$_2$ inhibitor darapladib did not show any significant beneficial effects in preventing the primary end-point of cardiovascular (CV) death, myocardial infarction, or stroke.[23] Interestingly, the above-mentioned association of the Lp(a) polymorphism with AV calcification was independent of coronary artery calcification and clinical CAD,[21] lending additional support to a differential pathophysiology between atherosclerosis and AS. Furthermore, a recent study has strengthened the implication of the Lp(a)/Lp-PLA$_2$ pathway in AS by showing that either Lp-PLA$_2$ expression or activity in human stenotic AVs correlated with valve weight, stenosis severity, and valvular calcium content.[24] In the latter study, lysophosphatidylcholine, which is a product of oxidised phospholipid metabolism by Lp-PLA$_2$, induced mineralisation of VICs, an *in vitro* model of valvular calcification.[24] Although an appealing perspective, the potential impact of Lp-PLA$_2$ inhibitors on AS currently remains to be established.

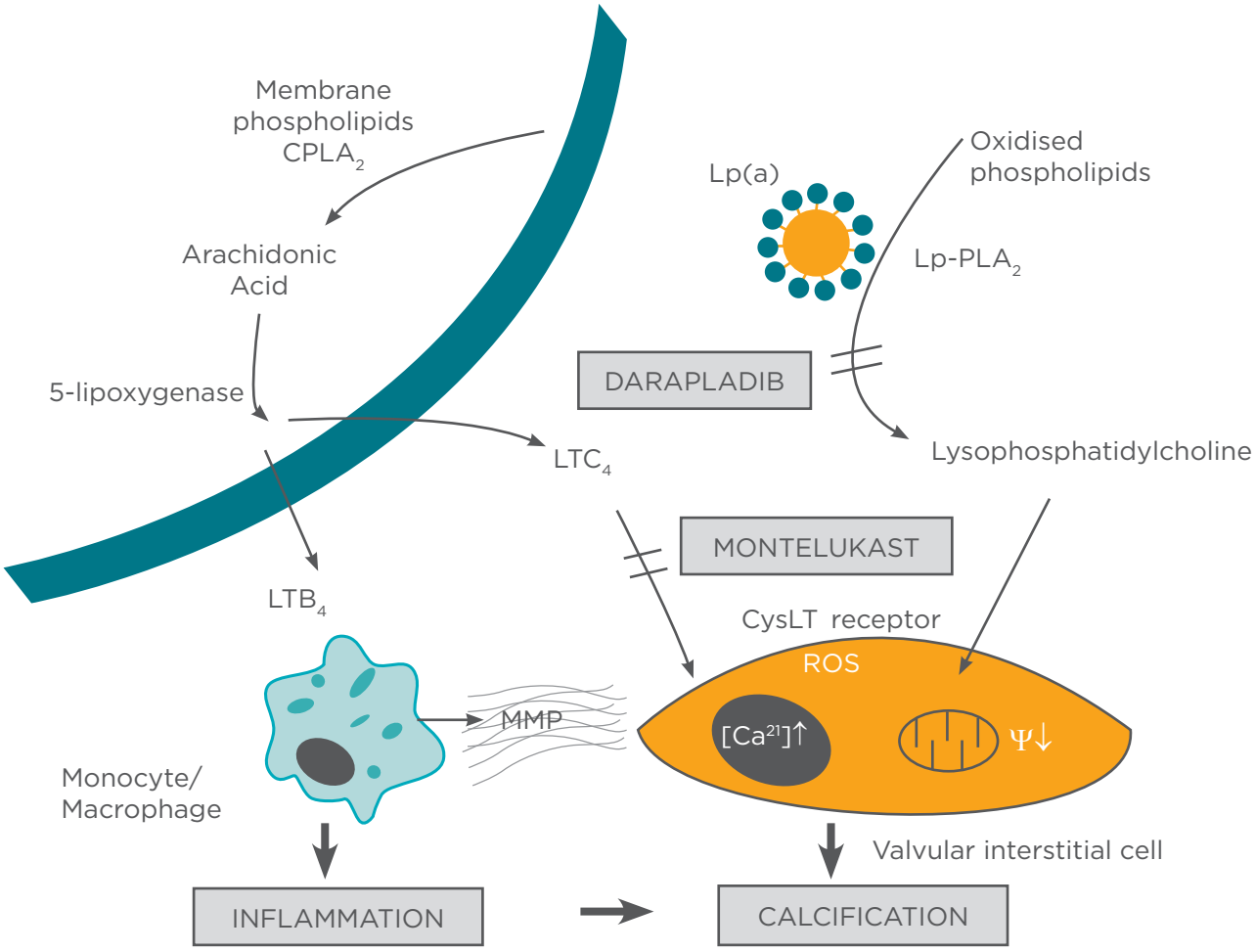

Figure 2: Lp(a), phospholipases and lipid mediators of inflammation in AS.
Membrane phospholipids are metabolised by cytosolic PLA$_2$ into lipid mediators of inflammation, such as leukotrienes, which induce recruitment and activation of inflammatory cells, and activate intracellular pathways in valvular interstitial cells associated with calcification. On the other hand, oxidised phospholipids hydrolysed by Lp-PLA$_2$ yield lysophosphatidyl choline, which also stimulates a calcification process through similar pathways in valvular interstitial cells. The effects of leukotrienes can be blocked by leukotriene receptor antagonists, such as montelukast, whereas darapladib is an Lp-PLA$_2$ inhibitor.
AS: aortic stenosis; Lp: lipoprotein; c: cytosolic; PLA$_2$: phospholipase A$_2$; LT: leukotriene; MMP: matrix metalloproteinase; ROS: reactive oxygen species; Ca: calcium; Ψ: mitochondrial membrane potential.

TARGETING INFLAMMATION

Also intracellular metabolism of phospholipids by means of another phospholipase, namely the cytosolic Group 4A phospholipase A2, ($cPLA_2$) may represent key pro-inflammatory stimuli in AS (Figure 2). Arachidonic acid, which is released from cell membrane phospholipids by means of $cPLA_2$ metabolism, serves as the substrate for the production of potent lipid mediators of inflammation. Arachidonic acid metabolism leads to the production of prostaglandins and thromboxanes through the cyclooxygenase pathway, whereas the 5-lipoxygenase enzyme catalyses the formation of leukotrienes, as depicted in Figure 2. Interestingly, the expression levels of the leukotriene synthesising enzyme 5-lipoxygenase in the thickened portion of human stenotic AVs are significantly associated with the severity of AS, as determined by echocardiographic criteria.[6] This overexpression results in an increased leukotriene production in AVs,[6] which was recently shown to correlate with valvular calcium content.[25] The role of leukotrienes in recruitment and activation of inflammatory cells is well established, and is linked to chemotaxis, protease release, as well as activation of proinflammatory pathways, such as NF- κB induction, which are all hallmarks of AS.[26] In addition, human VICs express the $CysLT_1$ subtype of leukotriene receptors, and leukotriene C_4 induces nuclear calcium signalling, increased production of reactive oxygen species, and a reduction of the mitochondrial membrane potential in these cells (Figure 2).[6,27] The latter processes may be of importance for the concomitant induction of osteogenic proteins and increased calcification observed *in vitro* after leukotriene stimulation.[6]

Given the important role of inflammation in the pathophysiology of AS, anti-inflammatory treatment could represent a potential therapeutic avenue to explore in search of medical treatments of AS. To obtain a rapid transfer of knowledge from bench to bedside, it is of particular interest that anti-leukotrienes today are in clinical use for the treatment of asthma. We recently extrapolated the anti-inflammatory effect exerted in asthma by the leukotriene receptor antagonist montelukast, to CV disease. In a phamacoepidemiological study of a population-based cohort, montelukast was associated with decreased CV risk, preventing the recurrence of either myocardial infarction or stroke.[28] Taken together, these observations suggested a generalisability of the anti-inflammatory effects of leukotriene modifiers beyond pulmonary disease. To test the applicability of the hypothesis that anti-leukotrienes have the potential to be protective for the development of AS, we have analysed the incidence of AS in relation to the use of montelukast in a nationwide population-based cohort of approximately 7 million subjects. With the limitations of integrating only administrative registry data and a short follow-up time (3.5 years), this analysis indicated a trend towards reduced incidence of AS associated with montelukast use, which however, did not reach statistical significance.[29] However, these results could potentially open up the design of future interventional studies targeting the leukotriene pathway in AS.

BISPHOSPHONATES (BPS)

Osteoclasts in AS

As mentioned above, valvular osteoclasts were recently identified in human AS.[11] Activation of RANK (receptor activator of nuclear factor kappa B) which is expressed on the surface of osteoclasts (Figure 1) by the RANK ligand (RANKL) causes release of proteases such as MMP-9, cathepsin K, and tartrate-resistant acid phosphatase. In contrast, osteoprotegerin (OPG), a soluble receptor which is part of the TNF receptor family, binds to RANKL, and hence, blocks its interaction with the RANK (Figure 1). The expression of the RANKL/RANK/OPG pathway has been demonstrated in AVs[10] which reinforces the notion of osteoclast activation in AS. Paradoxically, these osteoclasts do not seem to reduce valvular calcifications, but are rather associated with the progression of calcification.[11] This is radically opposed to other inflammatory diseases, such as periodontal bone loss due to a stimulation of osteoclasts by inflammatory mediators. Nevertheless, there may be a disjunction or malfunction of bone resorption, while the release of proteolytic enzymes from osteoclasts remains intact. The net effects of a stimulation of the osteoclasts would, in that case, be an increase in valve remodelling and an accelerated calcification.

Observational Studies of BPS in AS

BPS are analogues of pyrophosphate prescribed to prevent and treat osteoporosis. Their mechanisms of action are complex and not completely understood, but involve direct inhibition of osteoclasts. Since activation of osteoclasts may be deleterious in AS, BPS could potentially have

beneficial effects on AS progression. A number of retrospective studies have been reported in which the echocardiographic progression of AS has been followed according to BPS exposure, as shown in Table 2. Although the initial studies indicated that BPS use was associated with a decrease in AS progression (measured as either aortic valve area [AVA] or mean pressure gradient; Table 2),[30-32] the most recent and largest cohort did not reveal any significant differences in echocardiographic AS progression (AVA, P_{mean} [mean AV gradient], P_{max} [maximal pressure gradient]), valve replacement surgery or overall survival between BPS-treated and non-treated subjects with mild-to-moderate AS.[33] There are several differences between the studied populations, in terms of age, sex, and the proportion of BPS-treated subjects, which may account for the differential results reported (Table 2). In line with the above discussion on statin treatment in AS, the degree of AS at inclusion may have been decisive also in these studies for the possibility of detecting differences in stenosis progression (Table 2). The latter notion is supported by the results reported in the study by Sterbakova et al.[31] in which the annualised mean gradient change was lower in BPS-treated compared with the untreated patients with mild AS, whereas no effects of BPS treatment were observed in patients with moderate-to-severe AS.

Adding even more complexity to the role of BPS in this context, a CT study of 3,710 women from the community-based Multi-Ethnic Study of Atherosclerosis (MESA) reported that BPS exhibited age-dependent effects on CV calcification. First, AV calcification, defined as any calcified lesion within the AV leaflets, was more prevalent in BPS users compared with unexposed subjects.[34] Second, whereas BPS use was associated with increased AV calcification in women <65 years of age, a trend towards lower prevalence of AV calcification was reported in women ≥65 years.[34]

Table 2: Studies of BPS in AS.

Reference	Study population	Results
Skolnick et al.[29]	N=55 AVA 1.4 cm^2 2.4 years follow up Mean age 82 years 75% women	22% on BPS AVA ↓ 0.2 cm^2 (NT) AVA ↓ 0.1 cm^2 (Osteoporosis treatment; p=0.025)
Sterbakova et al.[30]	N=103 P_{mean} 33 mmHg 2.4 years follow up Mean age~70 years 51% women	27% on BPS P_{mean} ↑ 2 mmHg (NT) P_{mean} ↓ 0.3 mmHg (BPS; p=0.007)
Innasimuthu et al.[31]	N=76 AVA 0.6–2 cm^2 1.9 years follow up Mean age≈80 years 42% women	11% on BPS AVA ↓ 0.2cm^2 (NT) AVA ↑ 0.1 cm^2 (BPS; p=0.001)
Aksoy et al.[32]	N=801 AVA 1.0-2.0 cm^2 Mean age 76 years 100% women	39% on BPS No significant difference between BPS and NT in the rate of change in AVA, P_{mean} or P_{max}
Elmariah et al.[33]	N=3,710 CVD-free community cohort Mean age 63 years 100% women	5.8% on NC-BPS <65 years AC ↑ by NC-BPS ≥65 years AC ↓ by NC-BPS

AS: aortic stenosis; BPS: bisphosphonates; AVA: aortic valve area; P_{mean}: mean aortic valve gradient; NT: non-treated; P_{max}: maximal pressure gradient; CVD: cardiovascular disease; NC-BPS: nitrogen-containing bisphosphonates; AC: aortic calcification.

CONCLUSION

The molecular and cellular pathophysiology of AS is an active and complex process with components of inflammation, valvular remodelling, dystrophic calcification, oxidative stress, apoptosis, and heterotopic ossification (Figure 1). These pathways contain several potential targets for medical treatment, as has been exemplified above. For example, although statins appear to prevent calcification *in vitro*, the notion of targeting atherogenic lipids by means of statin treatment in AS has been challenged by the negative results of RCTs (Table 1). However, other lipid pathways, such as Lp(a) and Lp-PLA_2 have recently emerged in the context of AV calcification and AS (Figure 2). Furthermore, lipid mediators of inflammation, such as leukotrienes (Figure 2) may be interesting targets for future studies, as is also other anti-inflammatory treatments. Finally, whereas results of studies with BPS have generated contradictory results (Table 2), more specific targeting of calcification pathways activated in the AV could potentially be anticipated. Possible synergies between these pathways should also be considered in view of evaluating combination therapies. In conclusion, further mechanistic studies are needed to better understand the pathophysiology of AS and to lead to new therapeutic strategies for the prevention, or at least the delay, of either surgical or transcatheter valve implantations.

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