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Fibroblast Growth Factor 21 Mimetics for Treating Atherosclerosis

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Fibroblast growth factor 21 (FGF21) is an atypical member of the FGF family. Acting in an endocrine fashion, it increases glucose uptake, modulates lipid metabolism, and sensitizes insulin response in metabolically active organs, including the liver and adipose tissue. Emerging evidence shows a strong correlation between circulating FGF21 levels and the incidence and severity of atherosclerosis. Animal studies have demonstrated a beneficial role of FGF21 in protecting against aberrant lipid profile, while recent development in FGF21 mimetics has provided further insight into the lipid-lowering effects of FGF21 signaling. The present review summarizes the physiological roles of FGF21, and discusses major breakthroughs and limitations of FGF21 mimetic-based therapeutic strategies for treating atherosclerosis.

Keywords: Fibroblast growth factor 21; Atherosclerosis; Dyslipidemia

INTRODUCTION

Fibroblast growth factor 21 (FGF21) is an atypical member of the FGF superfamily that exerts pleiotropic effects on metabolic regulation [1]. Since FGF21 lacks the classic heparin-binding domain which is crucial for binding to cognate FGF receptors (FGFRs) [1], it requires the presence of a co-receptor, β-klotho, for effective receptor docking [2]. FGF21 is able to enter the circulation without non-specific binding to heparin sulphate proteoglycan and function as an endocrine factor in target organs [1]. While there is mounting evidence showing the beneficial effects of FGF21 on obesity and diabetes in humans and animals [3,4], the physiological roles of FGF21 in atherosclerosis is gaining increasing attention only recently. This review summarizes the role of FGF21 in atherosclerosis development, and highlights some recent findings on the therapeutic potential of FGF21 mimetics in treating atherosclerosis.

SIGNALING CASCADE OF FGF21

FGFRs are a family of single transmembrane protein consisting of an extracellular ligand-binding domain and an intracellular tyrosine kinase domain [1]. FGFR1c, a splice variant of the FGFR1 subtype, is responsible for the majority of the physiological actions of FGF21 in the presence of β-klotho [5,6]. Although FGFRs are widely expressed in the body, the expression of β-klotho is largely restricted to the liver, pancreas, and white and brown adipose tissue [7]; hence, giving rise to tissue specificity of FGF21 signaling. The direct binding between FGF21 and FGFR/β-klotho leads to the phosphorylation of downstream

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targets, including FGFR substrate 2 (FRS2), protein kinase B (Akt), sirtuin 1 (Sirt1), glycogen synthase kinase 3 (GSK3), Raf, and signal transducer and activator of transcription 3 (STAT3), as well as a rapid rise in intracellular calcium [8,9], which subsequently promote the expression of target genes involved in glucose and lipid metabolism [10].

PHYSIOLOGICAL ROLE OF FGF21

FGF21 serves as a major sensor for metabolic stresses, including starvation, overfeeding, and cold exposure [11]. Its expression is markedly induced by peroxisome proliferator-activated receptor α (PPARα) in the liver during fasting [12], from where it promotes hepatic gluconeogenesis via a hypothalamus-pituitary-adrenal axis to maintain glucose homeostasis [13]. In adipocytes, FGF21 forms a feed-forward regulatory loop with PPARγ [14], a key regulator of adipogenesis [15], whereby PPARγ promotes the transcription of FGF21, and FGF21 sustains PPARγ activity by preventing its sumoylation [16]. FGF21 is responsible for the thiazolidinedione-mediated glucose uptake and adipogenesis, and the consequent improvement in insulin sensitivity and lipid storage [16]. FGF21 increases glucose uptake by up-regulating the expression of glucose transporter 1 (GLUT1) through the synergistic actions of serum response factor (SRF) and Ets-like protein 1 (Elk-1) [17]. In addition, it directly increases the expression and secretion of adiponectin, a potent insulin-sensitizing adipokine, which in turn contributes to alleviation of obesity-associated hyperglycemia, insulin resistance and hepatic steatosis [18]. Under cold conditions, the expression of FGF21 is up-regulated in both white and brown adipose tissues [19], where it drives adaptive thermogenesis, at least in part through increasing the protein levels of PPARγ coactivator 1α (PGC-1α), a major regulator of the browning machinery [20].

ANTI-DYSLIPIDEMIC AND ANTI-ATHEROSCLEROTIC PROPERTIES OF FGF21

Recent clinical studies have provided mounting evidence for a critical role of FGF21 in the development of atherosclerosis-related diseases (Tables 1, 2). Elevated serum FGF21 levels is independently associated with total cardiovascular events [21], coronary heart disease [22], atherosclerosis in carotid arteries [23,24] and in the extremities [25], and arterial stiffness [26]. It is also predictive of cardiovascular events and mortality in type 2 diabetic patients [27]. Moreover, FGF21 strongly correlated with a number of independent risk factors for atherosclerosis, including obesity [28], type 2 diabetes [29,30], hypertension [31], non-alcoholic fatty liver disease [32-34], diabetic nephropathy [35], chronic kidney disease [36], and dyslipidemia [22,37]. In particular, serum FGF21 correlated positively with total cholesterol and triglyceride [37], and negatively with high density lipoprotein cholesterol (HDL-C) and apolipoprotein A1 (apoA1) [22]. Genetic association studies also identified a single nucleotide polymorphism in FGFR2 gene showing replicable associa-

Table 1. Associations between FGF21 and Atherosclerotic Risk Factors

Risk factor	Result	Reference
Obesity	BMI correlated positively with serum FGF21	[28]
T2DM	Elevated plasma FGF21	[29]
	Elevated plasma FGF21 predicted diabetes development	[30]
Hypertension	Independently associated with elevated serum FGF21	[31]
Dyslipidaemia	Serum FGF21 correlated positively with serum TG	[37]
	Serum FGF21 correlated positively with total cholesterol	[37]
	Serum FGF21 correlated negatively with HDL-C	[22]
	rs2071616 SNP in FGFR2 gene was associated with LDL-C	[38]
NAFLD	Elevated hepatic FGF21 expression	[32]
	Elevated serum FGF21	[33,34]
Diabetic nephropathy	Elevated baseline serum FGF21 was associated with and predicted decline of renal function	[35]
CKD	Elevated serum FGF21	[36]

FGF21, fibroblast growth factor 21; BMI, body mass index; T2DM, type 2 diabetes mellitus; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; SNP, single nucleotide polymorphism; FGFR2, FGF receptor 2; LDL-C, low density lipoprotein cholesterol; NAFLD, non-alcohol fatty liver disease; CKD, chronic kidney disease.

Table 2. Associations between FGF21 and Cardiovascular Diseases

CV end-point	Result	Reference
CV mortality	Elevated serum FGF21 predicted combined morbidity and mortality in T2DM	[27]
Total CV outcomes	Elevated plasma FGF21	[21]
CHD	Elevated serum FGF21	[22]
Carotid artery IMT	Elevated serum FGF21	[23,24]
Arterial stiffness	Brachial-ankle pulse wave velocity independently associated with serum FGF21	[26]
LEAD	Elevated serum FGF21	[25]

FGF21, fibroblast growth factor 21; CV, cardiovascular; T2DM, type 2 diabetes mellitus; CHD, coronary heart disease; IMT, intima-media thickness; LEAD, lower extremity atherosclerotic disease.

Table 3. Selected List of FGF21 Mimetics with Reported Lipid-Lowering Properties

Name	Company	Structural feature/Modification	Lipid-lowering effect	Reference
R1MAb	Genentech	β-Klotho-independent agonistic mAb against FGFR1b/c	↓ Hepatic TC & TG; ↓ serum TC & NEFA (*db/db* mice)	[43]
Fc-FGF21(RG)	Amgen	L98R (↓ aggregation); P171G (↓ proteolysis); Fusion to Fc (↑ half-life)	↓ Serum TC & TG (DIO mice) ↓ Fasted serum TG (DIO monkeys)	[45]
LY2405319	Lilly	L118C, A134C (disulphide bridge); S167A (↓ glycosylation in yeast); HPIP deletion (↓ proteolysis)	↓ Serum TG, TC & VLDL-C; ↑ serum HDL-C (diabetic rhesus monkeys) ↓ Serum TG, TC, LDL-C & VLDL-C; ↑ serum HDL-C (humans)	[48,51]
PF-05231023	Pfizer	A129C (linkage to CVX-200); CVX-200 conjugation (↑ half-life)	↓ Serum TG & VLDL-C; ↑ serum HDL-C (obese cynomolgus monkeys) ↓ Serum TG, TC & LDL-C; ↑ serum HDL-C (humans)	[53]

FGF21, fibroblast growth factor 21; R1MAb, FGFR1 with monoclonal anti-FGFR1 antibody; mAb, monoclonal antibody; FGFR1, FGF receptor 1; TC, total cholesterol; TG, triglyceride; NEFA, non-esterified fatty acid; Fc, antibody constant domain; DIO, diet-induced obesity; HPIP, histidine-proline-isoleucine-proline; VLDL-C, very low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; CVX, CovX-body.

tion with low density lipoprotein cholesterol (LDL-C) [38].

The lipid-lowering properties of FGF21 in atherosclerosis have been demonstrated in atherosclerosis-prone apoE$^{-/-}$ mice [39]. Lack of FGF21 in these mice led to marked exacerbation of atherosclerotic plaque formation and reduced lifespan [39]. Mechanistic studies showed that, in addition to acting through adiponectin-dependent mechanisms which inhibit local vascular inflammation and neointima formation [39], FGF21 attenuates hypercholesterolemia by suppressing the activity of hepatic sterol regulatory element-binding protein-2 and reducing cholesterol synthesis [39]. Hepatic deficiency of β-klotho largely compromised the cholesterol-lowering effects of FGF21, for which FGFR2 was responsible [39]. In line with this, FGF21 suppresses the expression of stearoyl-coenzyme A (CoA) denaturase 1 and 3-hydroxy-3methylglutaryl-CoA reductase in the liver in mice, which are involved in lipogenesis and cholesterol synthesis respectively [40]. Therefore, the liver appears to be a major target of FGF21 in suppressing cholesterol and lipid levels. The lipid-lowering effects of FGF21 have been consistently demonstrated in mice [18,41]. In diabetic monkeys, the administration of recombinant FGF21 does-dependently improved the blood lipid profile, as indicated by significantly reduced triglyceride, total cholesterol, LDL-C and very low density lipoprotein cholesterol (VLDL-C), as well as increased HDL-C [42].

THERAPEUTIC POTENTIAL OF FGF21 MIMETICS IN TREATING ATHEROSCLEROSIS

FGF21 mimetics can be roughly categorized into FGF21 analogues and activating antibodies against FGFR/β-klotho. A number of animal [43-50] and human [51,52] studies have

shown superior efficacy of FGF21 mimetics compared with native FGF21 in terms of half-life and resistance against aggregation and *in vivo* degradation. While most of these studies focused on improving insulin sensitivity and β-cell function in obesity and diabetes, some studies reported encouraging outcomes in combating proatherosclerotic lipid profiles (Table 3).

R1MAb (monoclonal anti-FGFR1 antibody), a phage-derived agonistic monoclonal antibody specific for FGFR1 with nanomolar affinity, was the first FGF21 mimetic identified to possess lipid-lowering properties at least in mice [43]. Administration of R1MAb to genetically diabetic *db/db* mice caused significant reduction in hepatic cholesterol and triglyceride, as well as serum cholesterol and non-esterified fatty acids [43]. The beneficial effects conferred by R1MAb were similar to those by native FGF21, which depend on normal functioning of adipose tissues [43]. Remarkably, a single injection of this monoclonal antibody exhibited sustained activity for more than 30 days [43]. Antibody-based FGF21 mimicry is therefore a promising option for long-term treatment of dyslipidemia and atherosclerosis.

Veniant et al. [45] generated a long-acting FGF21, Fc (antibody constant domain)-FGF21(RG), by fusing an Fc motif to a recombinant human FGF21 containing two structurally-stabilizing mutations. In obese mice, Fc-FGF21(RG) displayed markedly improved pharmacokinetics compared with native FGF21, as its effects on reducing serum cholesterol and triglyceride, when administered at 2.3 mg/kg every 5 days, was comparable to that of human recombinant FGF21 (hrFGF21) administered twice daily at 1.0 mg/kg [45]. Further, Fc-FGF21(RG) significantly reduced serum triglyceride in fasted or fed rhesus monkeys [45]. It was, however, surprising that neither Fc-FGF21(RG) nor hrFGF21 reduced cholesterol in these obese monkeys, which was demonstrated previously by Kharitonenkov et al. [42].

LY2405319 is a long-acting FGF21 which has an additional stabilizing disulphide bond and lacks the proteolysis-prone N-terminus [48]. The lipid-lowering properties of LY2405319 were more comprehensively investigated in both diabetic rhesus monkeys [48] and humans [51]. Daily administration at 3 mg/kg was sufficient for a significant improvement in circulating lipids in rhesus monkeys as soon as 2 weeks into treatment [48]. Levels of triglyceride, total cholesterol and VLDL-C were markedly reduced, while that of HDL-C was elevated significantly. LDL-C was modestly reduced by LY2405319 treatment [48]. Similarly, a randomized, placebo-controlled and double-blinded trial involving obese patients with type 2 diabetes showed positive and rapid effects on these lipids with daily LY2405319 treatment, being observable by as early as 2 days, and reaching maximal effect within 1 to 3 weeks, except that a higher dose (10 or 20 mg/kg) was required to have significant impact on total cholesterol and LDL-C levels [51].

Another long-acting FGF21, PF-05231023, was engineered by site-specific covalent conjugation of two hrFGF21 molecules to the Fab motif of a scaffold antibody, CVX-200 [49]. This complex was shown to have up to 70-fold increase in half-life compared with native FGF21 [49]. Pharmacokinetic and pharmacodynamics evaluation also suggested a bi-weekly intravenous (IV) delivery regimen in humans [52]. A subsequent study in obese cynomolgus monkeys showed that bi-weekly IV administration at 10 mg/kg significantly improved the lipid profile. Specifically, triglyceride was reduced by around 70% by 8 days, whereas for lipoproteins, there was a 74% reduction from baseline in the VLDL-C fraction and a 27% increase in the HDL-C fraction after 4 weeks [53]. Consistent with previous reports [48,52], the LDL-C fraction was less sensitive to FGF21 mimetics as no significant difference was observed between vehicle and treatment groups [53]. PF-05231023 also exhibited additive lipid-lowering effects in overweight or obese humans with type 2 diabetes who were on stable dose of metformin in a phase 1b trial [53]. Bi-weekly IV dosages at 100 and 140 mg/kg effectively reduced triglyceride, total cholesterol, and LDL-C, and increased HDL-C by as early as day 8 [53]. Together, these clinical studies demonstrate high therapeutic potential of FGF21 mimetics in the treatment of atherosclerosis through reversing dyslipidemia, in addition to its beneficial effects on other conditions which promote atherosclerosis including obesity [51,53], hyperinsulinemia [51], and hypoadiponectinemia [51,53].

It is, however, noteworthy that the administration of FGF21 mimetics in humans was not without adverse events [51-53]. FGF21 has been discovered for less than two decades, and its functional role in many physiological aspects is still poorly understood. More comprehensive functional characterization of FGF21 in different organs and physiological systems is needed to ensure minimal harm is introduced during treatments. Its involvement in bone health, for instance, remains unclear [54,55]. Furthermore, novel approaches to sensitize FGF21 signaling through modulation of FGFR/β-klotho complex or downstream players should be explored. The introduction of standardized guidelines for FGF21 mimetics dosing will also be beneficial for rigorous evaluation of FGF21-targeted therapies.

CONCLUSIONS

FGF21 has recently emerged to be a major regulator of glucose

and lipid metabolism that exerts pleiotropic effects in multiple target organs, including the liver and adipose tissue. Although most of the early studies on FGF21 focused on glucose metabolism and insulin actions, recent evidence strongly suggests a critical role for FGF21 in modulating lipid and lipoprotein metabolism. FGF21 has been identified to be a predictive and prognostic biomarker of atherosclerotic risk factors and cardiovascular diseases. The therapeutic potential of FGF21-targeting approaches in treating atherosclerosis has been demonstrated by studies on several FGF21 mimetics, which possess superior pharmacological features compared with native FGF21 and exhibit prominent lipid-lowering effects in both animals and humans. Despite these initial favorable outcomes of FGF21 mimetics-based therapies, the evidence for their long-term efficacy, optimal therapeutic window and adverse side effects is relatively limited. Whether FGF21 mimetics can be routinely applied in treatment for atherosclerosis requires further investigations.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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