

A variant of FGF19 for treatment of disorders of cholestasis and bile acid metabolism

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Submitted Jan 13, 2015. Accepted for publication Feb 06, 2015.

doi: 10.3978/j.issn.2305-5839.2015.03.38

View this article at: <http://dx.doi.org/10.3978/j.issn.2305-5839.2015.03.38>

Fibroblast growth factor 19 (FGF19) is increasingly recognized as a key hormone regulating many functions. A recent publication by Luo *et al.* in *Science Translational Medicine* has advanced understanding of the diverse actions of FGF19 and has suggested how therapeutics with this protein may be optimized (1).

FGF19 in humans is a 24 kDa protein which is syntenic with FGF15 in rodents; both have similar tissue distribution and actions and are sometimes known as FGF15/19 (2). FGF15/19, together with FGF21 and FGF23, are members of the larger group of fibroblast growth factors and share the property of low heparin sulfate binding, which allows them to function as circulating endocrine molecules with effects on distant tissues. FGF15/19 has major effects regulating hepatic bile acid synthesis (3) and also acts on carbohydrate and lipid metabolism (4). FGF21 also has metabolic functions, and FGF23 is the key hormone regulating phosphate homeostasis. These effects are produced through FGF receptors (FGFR). FGF15/19 at physiological levels acts preferentially through FGFR4, which forms complexes with β -Klotho, and produces intracellular effects through kinase actions (2,5).

FGF19 is synthesized in the ileum in response to bile acid absorption. Bile acids bind to farnesoid X receptor (FXR) and potentially activate transcription of FGF15 in mouse intestine (3) and FGF19 in human ileal explants [reviewed in (6)]. FGF19 enters the portal venous circulation and inhibits new bile acid synthesis in the liver by decreasing CYP7A1 activity, thus providing negative feedback. Serum FGF19 increases after meals (7) and is much lower in patients who have had ileal resection or with inflammation in Crohn's disease (8-10). Impaired

regulation of bile acid synthesis by FGF19 has been proposed to be a causative factor in the common disorder known as primary bile acid diarrhea or idiopathic bile acid malabsorption (8). Furthermore, obeticholic acid, a semi-synthetic modified bile acid and a potent FXR agonist, stimulated FGF19 production and improved symptoms in these patients (11). A role for defective FGF15/19 in bile acid-induced diarrhea is further supported by evidence from mouse models with knock-out of the components of FGF15 system (*Fgf15*, *Fgfr4* and *Klb*) which have increased fecal bile acid loss, increased synthesis and a larger bile acid pool size. Additionally, severe diarrhea resulted in monkeys when FGF19 was neutralized with antibodies [reviewed in (6)]. Therapy to increase FGF19 actions seems to be a promising approach to treating this form of chronic diarrhea associated with excess bile acid production.

The metabolic actions of FGF19 also suggest therapeutic possibilities in obesity, diabetes and the metabolic syndrome (2,4,12). Obese subjects and those with nonalcoholic fatty liver (NAFLD) and/or metabolic syndrome may have lower median serum FGF19 levels (13), which increase rapidly after bariatric surgery (14). Obeticholic acid stimulates FGF19 and produced clinical and histological improvements in NAFLD patients (15).

Importantly, FGF19 may be beneficial in cholestatic disorders. FGF19 is secreted by the human gallbladder into bile and transcripts are greatly increased in the livers of patients with extrahepatic cholestasis with increased amounts of protein being found in blood (16). It has been suggested that the role of this induced FGF19 is to inhibit bile acid synthesis by CYP7A1 in an autocrine/paracrine fashion so preventing accumulation of toxic levels of bile

acids. After liver resection in mice, FGF15/19 was shown to modulate bile acid levels preventing toxic injury and improving survival. Liver growth, DNA synthesis and cell proliferation were increased (17).

Such proliferative actions have led to concern about the possible relevance of FGF19 for liver neoplasia in humans (18). Indeed, FGF19 and FGFR4 are co-expressed in a proportion of human liver, lung and colon tumors (19). Expression of FGF19 was associated with recurrence and a relatively poor prognosis in hepatocellular carcinoma (20). In a transgenic mouse model, overexpression of human FGF19 in skeletal muscle (to levels many hundred times those normally found) resulted in increased hepatocyte proliferation and hepatocellular carcinomas, and increased proliferation also occurred after injection of supraphysiological amounts (30 µg/mouse) of recombinant FGF19 (21).

To address these potential concerns, Luo and colleagues have modified the FGF19 molecule, and in an elegant series of experiments, show this is nontumorigenic but retains the beneficial actions on bile acid metabolism and in cholestasis. The modified FGF19, known as M70, has a five amino-acid deletion and three amino-acids substituted at the N-terminal region. M70 showed none of the liver tumor-inducing activity of FGF19 when these forms were expressed in mice via an adenoviral vector for 24 weeks. It should be noted that this system gives serum levels of FGF19 or M70 which were around 2 µg/mL, 10,000-fold higher than usual human serum levels (~0.2 ng/mL) (7,8). Expression of markers including glutamine synthase, Ki-67, α -fetoprotein and cyclins was increased by FGF19 but not by M70 in this model.

M70 retained the ability of FGF19 to inhibit bile acid synthesis through decreased activity of the key rate-limiting enzyme CYP7A1. This was confirmed over a range of doses, both in human primary hepatocytes and in mice injected with either protein. It was shown in two mouse models of cholestasis (one extrahepatic and one intrahepatic) to be protective when it was given for 5 days before bile duct ligation and for 4 additional days. Necrotic areas in the liver and markers of damage were reduced by both FGF19 and M70. Similar benefits were found in the α -naphthylisothiocyanate model of intrahepatic cholestasis. M70 was shown to normalize hepatic transporter transcripts which were deranged in the cholestasis models.

The M70 protein was further validated in phase I studies in normal human volunteers. In a randomized, double-blind, placebo-controlled trial, subcutaneous injections of M70 (3 mg/d) or placebo were given for 7 days. M70 suppressed serum levels of 7 α -hydroxy-4-

cholesten-3-one (C4) by over 95%, indicating marked suppression of new bile acid synthesis and this effect persisted for 24 h after dosing. Post-prandial serum bile acid levels were decreased. No serious adverse events or laboratory abnormalities occurred.

The implications of this translational study are far-reaching. A single daily dose of M70 appears safe and is effective at suppressing bile acid synthesis in humans. Important benefits were shown in mouse models of cholestasis. These findings suggest that injections of M70 (and perhaps native FGF19) could have therapeutic roles in adverse conditions of excessive bile acid production including, but not limited to, intrahepatic and extrahepatic cholestatic conditions, but also in intestinal disorders of bile acid diarrhea, either primary or that secondary to Crohn's disease. Particularly in this later condition, where ileum has been resected or is diseased, FXR agonists such as obeticholic acid may not be able to stimulate ileal FGF19 production sufficiently (10,11); injected replacement therapy may be preferable.

Any concerns about FGF19 promoting hepatocellular carcinoma in humans may not turn out to be realistic at physiological levels. The relationships between FXR and hepatocarcinogenesis are complex (22), and the critical interactions of FGF19 with FGFR4 appear different in humans to those in mice (23). However the development of M70, lacking any liver tumor-promoting effects in mice, appears to be a major advance which should lead to further clinical investigation and hopefully important therapeutic benefits.

Acknowledgements

Disclosure: JR Walters has been a consultant for NGM Biopharmaceuticals and Intercept Pharmaceuticals. RN Appleby has been supported by research funding from Intercept Pharmaceuticals.

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Cite this article as: Walters JR, Appleby RN. A variant of FGF19 for treatment of disorders of cholestasis and bile acid metabolism. *Ann Transl Med* 2015;3(S1):S7. doi: 10.3978/j.issn.2305-5839.2015.03.38