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Review

Farnesoid X receptor activation improves erectile dysfunction in models of metabolic syndrome and diabetes[☆]

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ABSTRACT

The metabolic syndrome (MetS) is an insulin-resistant state characterized by a cluster of cardiovascular risk factors, including abdominal obesity, hyperglycemia, elevated blood pressure and combined dyslipidemia. In this review, we discuss the potential of farnesoid X receptor (FXR) agonists in the treatment of erectile dysfunction (ED), a multifactorial disorder often comorbid with MetS. FXR not only regulates lipid and glucose homeostasis but also influences endothelial function and atherosclerosis, suggesting a regulatory role for this hormone nuclear receptor in the cardiovascular complications associated with the MetS, including ED. MetS induces ED via several mechanisms, and in particular through endothelial dysfunction in penile vessels. In a high-fat diet rabbit model of MetS, a 3-month treatment with the potent and selective FXR agonist INT-747 restores endothelium-dependent relaxation in isolated cavernous tissue, normalizing responsiveness to acetylcholine and to electrical field stimulation. Accordingly, eNOS expression in the penis is greatly up-regulated by INT-747 treatment. Experiments in a rat model of chemically-induced type 1 diabetes further demonstrate that INT-747 treatment preserves erectile function induced by electrical stimulation of the cavernous nerve. These results add a new facet to the pleiotropic activities mediated by FXR, and reveal novel beneficial effects of FXR activation with potential clinical relevance. This article is part of a Special Issue entitled: Translating nuclear receptors from health to disease.

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1. Introduction

Erectile dysfunction (ED) is defined as the consistent inability to achieve and/or maintain an erection sufficient for satisfactory intercourse [1]. ED is a very common multidimensional disorder that has detrimental effects on sexual and reproductive activity, thereby impairing the quality of life of both the patient and his partner. A 2009 analysis of all population-based studies conducted in the US indicates that ED is indeed the most common endocrine disorder in men [2]. A European survey showed that ED affects almost 30% of men in an age-dependent manner [3]. ED results from a continuous spectrum of clinical factors, including physical illness (the organic component of ED), reaction to stress (the intrapsychic component of ED) and relationship difficulties (the relationship component of ED), which are often simultaneously present in ED patients and mutually concur in determining the disorder [4,5]. However, the most important risk factors for ED are associated to the impaired balance between contractant and relaxant mechanisms of

penile structures, resulting in arterial insufficiency and defective smooth muscle relaxation.

Thus, penile erection is essentially a neurovascular event initiated by sexual arousal, which stimulates release of nitric oxide (NO) at nerve endings in the penis resulting in the engorgement of the two sponge-like cavernous bodies (corpora cavernosa) with blood (Fig. 1). The NO produced through NO synthase, present in both endothelial cells and neurons, diffuses into smooth muscle cells (SMCs) and increases the formation of cyclic GMP (cGMP), which, through cGMP-dependent protein kinase G, promotes SMC relaxation by decreasing intracellular calcium levels. Relaxation of cavernous SMCs increases blood flow to cavernosal sinuses and mediates venous occlusion, resulting in penile engorgement and rigidity. The formation of cGMP is actively counteracted by a series of phosphodiesterases (PDEs), the most important of which is PDE5 [6]. In human corpora cavernosa, PDE5 alone accounts for the breakdown of the majority (~70%) of cGMP [7]. Conversely, sympathetic nerve activity is mainly responsible for maintaining SMCs in a contractile state. When released from nerve endings, norepinephrine binds to its cognate receptors. Receptor binding activates two separate pathways within the cells, one leading to the activation of rho kinase (ROCK) and the other causing increased levels of inositol triphosphate, which result in increased intracellular calcium levels and calcium sensitization. As soon as pro-contractile transmitters, as endothelin-1 (ET-1) or

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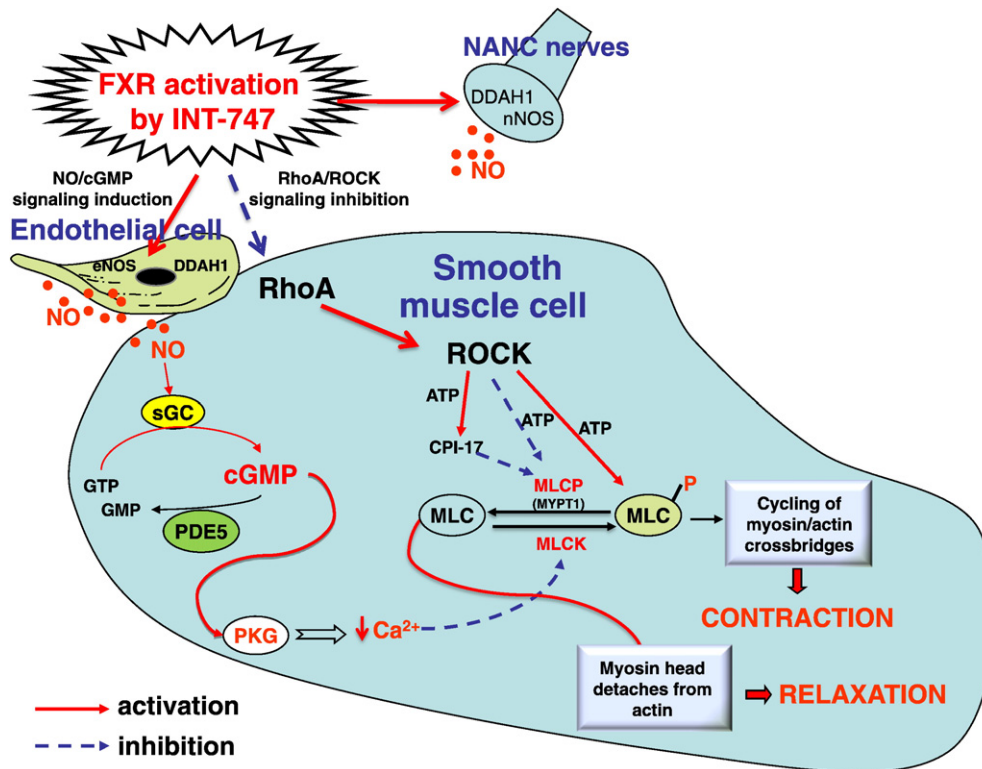


Fig. 1. FXR-mediated molecular mechanism underlying modulation of corpora cavernosa smooth muscle cell function. Following FXR activation by INT-747, induction of eNOS, nNOS and DDAH1 positively regulates NO release from endothelium and nerves leading to NO/cGMP-mediated smooth muscle cell relaxation. The inhibition of the pro-contractile RhoA/ROCK signaling by FXR activation further promotes corpora cavernosa smooth muscle cell relaxation and thereby erectile function. NANC, nonadrenergic noncholinergic; NO, nitric oxide; eNOS, endothelial NO synthase; nNOS, neuronal NOS; DDAH1, dimethylarginine dimethyl-aminohydrolase-1; sGC, soluble guanylate cyclase; GTP, guanosine triphosphate; GMP, guanosine monophosphate; cGMP, cyclic GMP; PKG, cGMP-dependent protein kinase; PDE5, phosphodiesterase type 5; MLC, myosin light chain; MLCK, MLC kinase; MLCP, MLC phosphatase; MYPT1, myosin phosphatase target subunit 1; ATP, adenosine triphosphate; ROCK, Rho-kinase; CPI-17, protein-kinase C-potentiated myosin phosphatase inhibitor.

noradrenaline (NA), bind to their excitatory receptors, RhoA, a small monomeric GTPase protein mainly localized in the cytosol in its RhoA-guanosine diphosphate (GDP) inactive form, exchange in an active guanosine triphosphate (GTP)-bound complex that translocates to the plasma membrane. Upon activation, RhoA-GTP anchors to the membrane through its geranylgeranyl pyrophosphate (GGPP) isoprenoid group and activates its downstream effectors, among which ROCK is the best characterized. The two described ROCK isoforms (ROCK1 or ROKb, and ROCK2 or ROKa) are serine-threonine kinases that are able to maintain the phosphorylated state of the myosin light chain (MLC) by phosphorylating and inhibiting the myosin phosphatase target subunit 1 (MYPT1) of MLC phosphatase, thereby promoting actin/myosin cross-bridging and contraction, independently of intracellular calcium levels [8,9]. Finally, RhoA/ROCK pathway plays a major role in maintaining the contractile tone in penile SMC, independently of intracellular calcium levels [5,8–11].

As briefly reviewed above, ED is a multifactorial disorder. However, organic factors are found in more than 80% of cases and include endocrine, neurogenic and arteriogenic causes [5,12,13]. In particular, endothelial dysfunction has been reported as the most important determinant of ED, and ED itself is now recognized as one of the earliest manifestations of a forthcoming cardiovascular disease (CVD) [14]. The “artery size hypothesis” indicates that ED, on average, occurs 3 years earlier than a subsequent CVD, because the smaller penile arteries reach critical narrowing, with insufficient blood flow, earlier than larger vessels [15,16]. Accordingly, the Prostate Cancer Prevention Trial reported that incident ED was associated with a hazard ratio of 1.25 [1.02–1.69] for subsequent cardiovascular events, during 9 years follow up after adjustment for confounders [17]. Hence, detecting an arteriogenic ED might help clinicians in preventing further CVD complications.

In most cases, the first-line pharmacological approach for ED is based on phosphodiesterase type 5 (PDE5) inhibitors, competitive inhibitors of cGMP for PDE5 [18]. Three oral PDE5 inhibitors are currently available: sildenafil, vardenafil, and tadalafil, whilst a number of newer agents are licensed in a few countries (udenafil, mirodenafil) and others (avanafil, lodenafil, SLX-2101) are currently under development. The efficacy, safety, and tolerability of PDE5 inhibitors have been recently reviewed [19]. However, a large number of men are unable to utilize oral treatments for ED. This is mainly due to the fact that 25–32% of ED-patients are non-responders to PDE5 inhibitors and some have contraindications to PDE5 inhibitors. These patients are shifted to second-line treatments, local and invasive therapy with vasoactive agents. Three main vasoactive drugs are currently used for intracavernosal injection (ICI) therapy of ED, namely prostaglandin E1 (PGE1), vasoactive intestinal polypeptide and phentolamine [19]. Most commonly reported adverse effects are penile pain, occurring in 50% of patients, and prolonged erections. Table 1 summarizes the main pharmacological therapies for ED currently used or under development.

2. Metabolic syndrome

Metabolic syndrome (MetS) is a diagnostic category, based on a cluster of risk factors (hyperglycemia/diabetes, abdominal obesity, hypertriglyceridemia, low HDL cholesterol and hypertension), which identifies subjects at high risk for forthcoming type 2 diabetes mellitus (T2DM) and CVD [20,21]. Any definition of MetS is, at present, largely arbitrary. In epidemiological studies, the National Cholesterol Education Program-Third Adult Treatment Panel criteria (NCEP-ATPIII) [22] are used frequently due to their simplicity, rather than to an intrinsic superiority [20]. MetS, according to the NCEP-ATPIII definition, is the

Table 1
Characteristics of pharmacological therapies for ED currently used or under development.

	Sildenafil	Vardenafil	Tadalafil	Udenafil	SLx-2101	Avanafil	Mirodenafil	Lodenafil
PDE5 inhibitors								
Formulation (mg)	25, 50, 100	5, 10, 20	10, 20	100, 200	5, 10, 20, 40, 80	50, 100, 200, 300	50, 100	20, 40, 80
<i>Pharmacokinetic properties</i>								
T_{max} (h)	1	1	2	1–1.5	1	0.33–0.52	1.25	1.2
C_{max} (fasting) (ng/ml) (100 mg)	450	20.9	378	416	No data	3992	No data	157
$T_{1/2}$ (h)	3–5	4	17.5	11–13	9–14	5.3–10.6	2.5	2.4
Advantages	Oral	Oral	Oral, longer duration of action	Oral, longer duration of action, greater selectivity	Oral, fast-onset, long-acting	Oral, fast-onset, short duration of action	Oral, effective and safe in men with ED concomitantly taking antihypertensive medications	Oral
Side effects	Headache, flushing, dyspepsia, occasional visual changes, nasal congestion, rare cardiovascular risks	Nausea, flushing, dyspepsia, nasal congestion, rare heart attack risk	Headache, flushing, dyspepsia, nasal congestion, back or girdle pain, priapism	Facial flushing, nasal congestion, ocular hyperemia, headache	No data	Flushing, headache, dizziness, nasal congestion, orbital pain	Facial flushing, headache, nausea, eye hyperemia	Headache, dyspepsia, rhinitis, flushing, color visual disorders
References	[19,90]	[19,90]	[19,90]	[19,91]	[19]	[19,92]	[19,93,94]	[19,95]
Intracavernosal prostaglandin E1								
Formulation	10 µg							
Advantages	Low cost							
Side effects	Penile pain; prolonged erection							
References	[19,96]							
Intraurethral prostaglandin E1								
Formulation	0.5, 1 mg							
Side effects	Urethral pain							
References	[19,97]							

presence of three or more of the following five factors: central obesity (waist circumference > 102 cm), elevated triglycerides (≥ 1.7 mmol/l or 150 mg/dl), elevated blood pressure (BP $\geq 130/85$ mm Hg), elevated fasting glucose (≥ 6.1 mmol/l or 110 mg/dl) and reduced HDL cholesterol (< 1.03 mmol/l 40 mg/dl) [22]. Moreover, all individuals receiving pharmacological treatment for hypertension, hypertriglyceridemia or low HDL cholesterol, and all individuals previously diagnosed with type 2 diabetes, should be considered as potentially afflicted by MetS. Besides the NCEP-ATPIII criteria, other definitions exist, and a most recent one from the International Diabetes Federation designates central obesity as an essential requirement [23]. Independent of its definition, MetS has been associated with a two-fold increase of 5- to 10-year risk of CVD, and a five-fold increase in risk for T2DM [21]. Despite this evidence, the clinical use of this category, and in particular its utility as a predictor of CVD, has been the subject of vigorous debate [24–29]. Given that individual MetS components are all cardiovascular risk factors, it is quite obvious that subjects with MetS have elevated CV risk. The hypothesis underlying the use of MetS in predicting CVD is based on the concept that its components could somehow have a synergistic effect on CV risk. Thus, the overall risk conferred by MetS should be greater than the sum of the risks associated with each of its individual components [30–32]. The evidence available, however, does not support this hypothesis. In a case-control study involving 393 early-onset coronary artery disease subjects, the prognostic information associated with the syndrome was not greater than the sum of its parts [33]. More recently, similar results were also reported in the INTERHEART study, a case-control study on the incidence of acute myocardial infarction involving more than 26,000 subjects in 52 countries [34] as well as in the RIVANA study, a population-based study involving 880 individuals [35]. In addition, the Rancho Bernardo Study, a community-dwelling sample of 338 participants with an average age of 67 years did not report any association between MetS and CVD [36]. In line with these results, a recent review of 3459 patients who participated in 7 clinical trials monitoring coronary

atheroma progression with intravascular ultrasonography demonstrated that, although accelerated disease progression was observed in the presence of MetS, this was due to the presence of individual component risks rather than to the presence of the syndrome itself [37].

3. Erectile dysfunction and metabolic syndrome

A large body of evidence suggests that MetS is frequently associated with ED and unresponsiveness to PDE5 inhibitors [5,20,38–40]. The prevalence of ED in subjects with MetS ranges from 27% [41] to 80% [42] and it is strictly associated with the number of MetS components and with the endothelial function impairment [5,20,38–40]. Longitudinal data indicate that ED can even be a predictor of MetS in men with normal weight at baseline [43]. Hence, ED could be the first sign of MetS and one of the earliest manifestations of a forthcoming CVD [32,44]. Accordingly, we have recently reported results from an observational prospective cohort study, involving a consecutive series of almost 1700 ED subjects, with an 8-year follow-up in which we showed that severe ED and reduced penile blood flow almost double the risk of incident major cardiovascular diseases, even after adjusting for age and comorbidities [45]. ED, previously considered no more than a frustrating condition, should thus be regarded as a good opportunity to screen for the presence of ED-associated comorbidities, such as MetS and silent CVD.

The association among MetS, ED, and hypogonadism has been also well recognized and the presence of hypogonadism in men with MetS and ED is associated with a greater severity of symptoms of sexual dysfunctions, other than ED [5]. The specific mechanisms underlying these associations are not completely clarified, although insulin resistance has been recently considered the common pathogenetic link between ED, MetS and male hypogonadism [46]. Accordingly, in insulin-resistant individuals, such as those with MetS, insulin-induced production of nitric oxide is impaired [47]. Furthermore, insulin-

resistant states are associated with atherosclerosis, which could produce lesions on penile arteries, therefore inducing a reduction of penile blood flow. Consistent with these findings, we have previously demonstrated that HFD-induced MetS [48] and streptozotocin-induced diabetes in animal models [49,50] are associated with penile function impairment.

Several neurotransmitters and endothelial factors have been shown to control erectile function by modulating the penile vasculature and smooth muscle tone of corpora cavernosa. Among these factors, NO released by nerves and endothelium in the penis plays a crucial role in the initiation and maintenance of increased intracavernous pressure and penile erection, but the normal erectile function is warranted by a tight balance between relaxant and contractile factors. Pathological conditions that alter this balance, such as those associated with reduced function of nerves and endothelium and/or overactivity of RhoA/ROCK contractile signalling in the penis (i.e. aging, hypertension, smoking, diabetes and MetS), cause changing in penile tissue function resulting in impaired smooth muscle relaxation and erection [50–53]. In human penile cells, hyperglycemia per se promotes activation of RhoA/ROCK pathway [53]. Conversely, nitric oxide synthase (NOS) activity is decreased by MetS [48] and type 1 diabetes [50,53–56], which also increases penile fibrosis by altering the smooth muscle/fiber ratio [50,57].

4. Signaling via farnesoid X receptor regulates glucose and lipid metabolism, with protective effects on the vasculature

The farnesoid X receptor (FXR) is a nuclear receptor functioning as an endogenous sensor for bile acids [58]. FXR is expressed at high levels in liver, intestine, kidney and at lower levels in adipose tissues, and is most potently activated by the primary bile acid chenodeoxycholic acid [58]. Besides expression in hepatocytes and enterocytes [58], FXR immunolabeling was observed in glandular cells in the zona reticularis/fasciculate of the adrenal gland and in the tubular epithelial cells of proximal, distal and collecting tubules of the kidney [59]. FXR is also expressed in preadipocytes and mature adipocytes of white adipose tissue [60], in pancreatic β -cells [61], in different cells of the immune system [58], and in smooth muscle and endothelial cells of the vascular bed [62].

Activation of FXR is involved in maintaining not only bile acid homeostasis, but also in regulating the intricate network governing lipid, cholesterol, and energy homeostasis, transport and metabolism of fatty acids and triglycerides, and control of glucose homeostasis [58]. FXR activation can also regulate immune responses, exerting an overall anti-inflammatory effect [58]. FXR agonists have been shown to suppress hepatic fatty acid and triglyceride synthesis through down-regulation of SREBP1c, and increase hepatic fatty acid oxidation through up-regulation of pyruvate dehydrogenase kinase 4 (PDK4) [58]. In agreement with these findings, plasma cholesterol and triglyceride levels are increased in FXR-deficient mice [63], which also show impaired glucose tolerance and insulin resistance [60]. In addition, loss of FXR function is associated with decreased survival, increased severity of defects in lipid metabolism, and more extensive aortic plaque formation in a mouse model of atherosclerotic disease [64].

Based on its important role in multiple metabolic pathways; accumulating evidence indicates that FXR may represent a promising pharmaceutical target for the treatment of defined metabolic diseases [65,66]. In particular, considering its capacity to regulate lipid and glucose metabolism as well as the atherosclerosis process with direct actions on the arterial wall, FXR modulation may be instrumental in the global management of cardiovascular risk factors associated with the MetS [67].

4.1. INT-747, a potent and selective FXR agonist

The semi-synthetic chenodeoxycholic acid derivative 6 α -ethyl chenodeoxycholic acid (INT-747, obeticholic acid) is a selective and

potent FXR agonist originally described for its anti-cholestatic properties [68]. Biodistribution and mass balance studies have demonstrated that INT-747 is well absorbed and efficiently conserved in the enterohepatic circulation. A small proportion of the parent compound is found in plasma, where it is mainly present as glyco- or tauro-conjugate, depending on the species examined, and quantitation of INT-747 as well as its metabolites in rabbit serum and corpora cavernosa is ongoing. INT-747 has been shown to have profound regulatory effects on lipid and glucose metabolism [69]. INT-747 improves glycemia by increasing peripheral glucose uptake, enhancing glucose-stimulated insulin secretion, and inhibiting hepatic lipid synthesis and content while inducing lipid uptake by adipocytes [70,71]. INT-747 has been successfully tested in phase II clinical studies in patients with primary biliary cirrhosis, as well as in patients with T2D and non-alcoholic fatty liver disease [69].

Moreover, a potential new role for FXR in the pathogenesis of cardiovascular disease has been proposed, also via a direct action on the vasculature. FXR activation by INT-747 in smooth muscle and endothelial cells of the vascular bed leads to apoptosis and inhibition of vascular inflammation, with beneficial effects potentially preventing cell adhesion and thrombosis [72]. Notably, INT-747 down-regulates IL-1 β -induced iNOS and cyclooxygenase-2 expression, and inhibits IL-1 β -induced NF- κ B activation and iNOS expression, showing important anti-inflammatory effects in vascular cells [72]. Preclinical studies using FXR and ApoE double-knockout mice fed a high-cholesterol diet show increased tendency towards development of atherosclerotic lesions in comparison with single-knockout mice, suggesting that FXR activation might have a mechanistic implication in protecting against atherosclerosis. In ApoE^{-/-} mice administration of INT-747 reduces the formation of aortic plaque area by 95%, with a similar anti-plaque activity to rosiglitazone [73]. INT-747 treatment inhibits diet-induced renal lipid accumulation, inflammation, fibrosis, and proteinuria [74]. In addition, INT-747 prevents the development of type 1 diabetic nephropathy by decreasing proteinuria, glomerulosclerosis and tubulointerstitial fibrosis and modulating renal lipid metabolism, macrophage infiltration and renal expression of SREBPs, profibrotic growth factors and oxidative stress enzymes [75]. INT-747 inhibits also phosphate-induced mineralization and triglyceride accumulation in calcifying vascular cells and ameliorates chronic kidney disease-induced vascular calcification in 5/6 nephrectomized ApoE^{-/-} mice [76]. Overall, these findings suggest that FXR activation by potent and selective FXR agonists like INT-747 may open new attractive pharmacological approaches for the treatments of MetS-related vascular dysfunction, including ED.

5. FXR activation by the selective agonist INT-747 improves ED in animal models of metabolic syndrome and diabetes

As reviewed above, growing evidence indicates that FXR regulates vascular homeostasis and remodelling [62], suggesting its potential role in the cardiovascular complications associated with the MetS [67]. Expression of FXR has been detected in both endothelial cells and smooth muscle cells involved in atherosclerotic plaque formation [58]. FXR-mediated regulation of endothelial NOS (eNOS) expression has been demonstrated in vascular endothelial cells [77], thus suggesting the involvement of this intracellular signalling in the positive regulation of vascular endothelial function. FXR might also interfere with endothelium-derived NO activity through modulation of serum asymmetric dimethylarginine (ADMA). ADMA is an endogenous NOS inhibitor and elevated plasma ADMA levels are associated with reduced NO synthesis in diseases related to endothelial dysfunction, including hypertension, hyperlipidemia, and diabetes mellitus [78,79]. In vitro studies have shown that FXR activation reduces ADMA formation by positively regulating the expression/activity of hepatic dimethylarginine dimethyl-aminohydrolase-1 (DDAH1), the major catabolic pathway of ADMA [80].

Vasodilatory effects are also suggested by the observation that FXR activation induces kininogen mRNA [81], which might result in increased local bradykinin in corpora cavernosa tissue thus resulting in vasodilation through the bradykinin receptor B2 signaling. Moreover, downregulation of ET-1 (with vasoconstrictive activity) in penile tissue could also contribute to the vasodilatory effect of FXR activation [82].

We have recently investigated the role of FXR activation on erectile function in two animal models of metabolic derangements: a rabbit model of high-fat diet (HFD)-induced MetS and a rat model of streptozotocin (STZ)-induced type 1 diabetes [83]. Both models are characterized by penile alterations [48,84]. FXR is expressed in penile tissue from different species, including humans, rats and rabbits, in both endothelial and smooth muscle cells of the vascular bed and cavernous spaces (Fig. 2). In addition, penile FXR expression is clearly up-regulated by chronic INT-747 treatment in both rabbit and rat models, thus suggesting not only that erectile tissue may be a target for FXR agonists, but also the existence of a positive feedback loop. Since chronic activation of FXR by non-steroidal FXR agonists (e.g. in mouse liver cells/tissue) does not necessarily result in upregulation of FXR mRNA, the effect observed in rabbit penile tissue after chronic INT-747 application appears novel and requires future investigations for clarifying whether other classical target tissues for INT-747, such as liver, show increased FXR expression.

In HFD rabbits, NO-mediated relaxation of isolated penile strips is hampered, as demonstrated by the blunted responsiveness to both acetylcholine and non-adrenergic non-cholinergic (NANC) nerve stimulation [48]. Accordingly, eNOS expression, along with DDAH1, was impaired in penile tissue from HFD rabbits. Chronic administration with INT-747 in HFD rabbits was able to partially restore penile responsiveness to both acetylcholine and NANC stimulation, and to increase both eNOS and DDAH1 expression in penile tissue [83]. This finding is consistent with the previously reported capacity of FXR triggering to directly enhance transcriptional activation of eNOS gene promoter in vascular endothelial cells [77] and of DDAH1 in the liver [80]. The expression of FXR in human and rabbit penile endothelial vascular cells, along with the almost complete normalization of the response to Ach and NANC stimulation, is consistent with an INT-747-mediated up-regulation of NO formation in corpora cavernosa.

Interestingly, FXR activation in penile tissue appears to be linked not only to the NO-mediated relaxation but also to the modulation of the pro-contractile RhoA/ROCK signaling. The relaxation induced by the NO-donor sodium nitroprusside (SNP), which bypasses NO formation, was more pronounced in HFD rabbits than in controls, and even more pronounced in INT-747-treated HFD rabbits. A possible explanation for this finding is that cGMP breakdown by PDE5 is reduced in HFD rabbits and not restored by INT-747, because of a MetS-induced down-regulation in PDE5 expression [48].

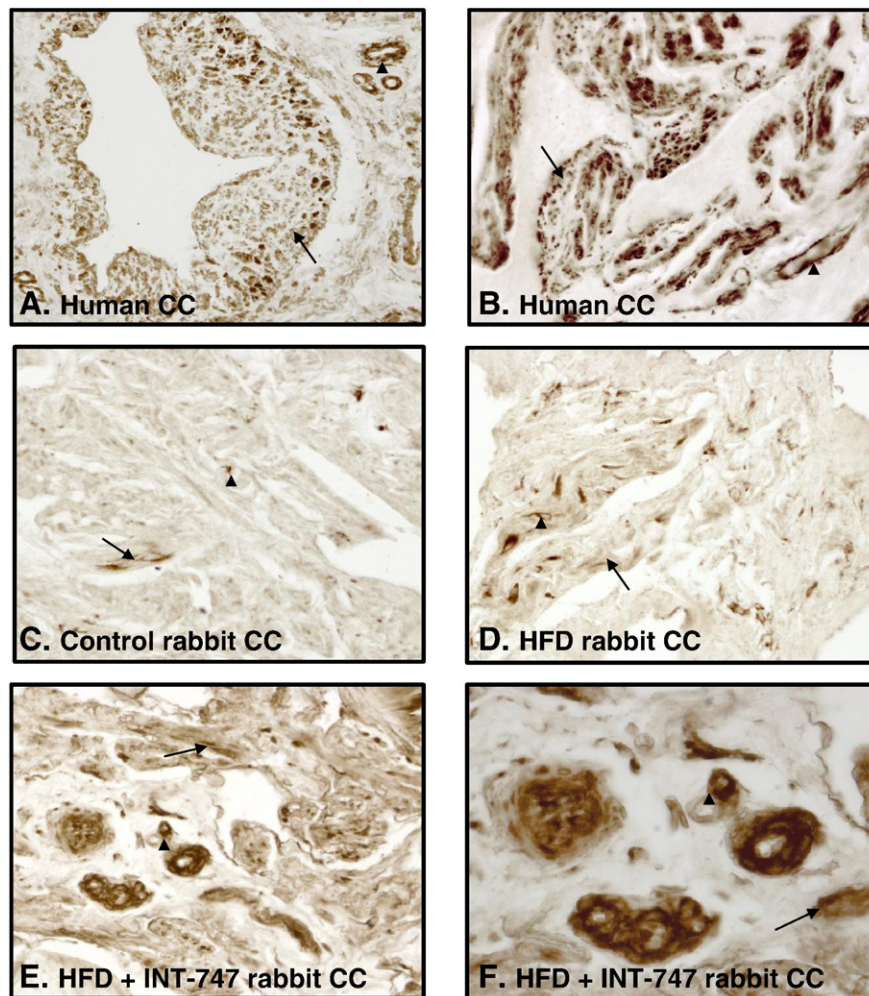


Fig. 2. Immunolocalization of FXR in human and rabbit corpora cavernosa (CC). Transverse sections of human CC (panels A and B). FXR immunopositivity is localized in smooth muscle (arrows) and endothelial cells (arrowheads) of the vascular bed and cavernous spaces (magnification $\times 20$). Transverse sections of CC from control (panel C, magnification $\times 20$), HFD (panel D, magnification $\times 20$) and HFD plus INT-747-treated rabbits (panels E and F, magnification $\times 20$ and $\times 40$, respectively). FXR-immunopositivity is localized in smooth muscle (arrows) and endothelial cells (arrowheads) of the vascular bed and cavernous spaces of rabbit CC. Adapted from Ref. 68 with permission.

Accordingly, in HFD rabbits, treated or not with INT-747, the *in vitro* responsiveness of penile strips to SNP was not further increased by blocking PDE5 with selective inhibitors, due to the reduced abundance of their target enzyme. However, the down-regulation of PDE5 did not fully explain the more pronounced relaxant effect of SNP in INT-747-treated rabbits, suggesting that FXR activation could target additional mechanisms, likely interfering with RhoA/ROCK signaling. Indeed, this pathway is hyperactive in corpora cavernosa of diabetic animal models and its inhibition causes penile smooth muscle relaxation, normalizing erectile function [50,53,85]. Studies in human fetal penile smooth muscle cells have demonstrated that high glucose (40 mM) is able to increase the membrane expression of active RhoA and MYPT-1 phosphorylation, indicating ROCK activity [53]. RhoA membrane fraction is significantly increased in penile tissue from HFD rabbits. This increase is prevented by *in vivo*, and even *in vitro* (in isolated penile smooth muscle cells) treatment with INT-747, which determines RhoA accumulation in the cytosol, therefore decreasing ROCK activity [83]. Accordingly, ET-1-stimulated MYPT-1 phosphorylation is decreased in penile cells by INT-747. Cellular events downstream RhoA/ROCK activation, such as cytoskeleton remodelling, expression of a motile phenotype, cell migration and expression of SM22 and α SMA are all down-regulated by *in vitro* exposure to INT-747 [83].

The beneficial effects of INT-747 in HFD-induced MetS were associated not only with up-regulation of NO transmission and inhibition of RhoA/ROCK pathway in penile tissue, but also with normalization of visceral adiposity and glucose intolerance [83]. Hence, in this animal model the efficacy of FXR activation on erectile function could be secondary to its metabolic effects. To rule out this possibility, a direct effect of INT-747 on erectile function has been tested in the rat model of STZ-induced type 1 diabetes, characterized by a marked hyperglycemia, in order to clarify whether INT-747 could improve erectile function independently of the effect on glycemia.

In this rat model, type 1 diabetes is associated with hyperactivity of RhoA/ROCK pathway, as demonstrated by the higher intracavernous pressure increase in response to intracavernous injection of the ROCK inhibitor Y-27632, in comparison to age-matched controls [50,53]. INT-747 dosing for 8 weeks completely restored penile responsiveness to Y-27632 in diabetic rats, suggesting *in vivo* down-regulation of the hyperactive RhoA/ROCK pathway. In addition, INT-747 completely normalized the depressed response of STZ-treated rats to electrical stimulation of the cavernous nerve. Interestingly, several of the STZ-induced metabolic alterations, such as hyperglycemia, hypercholesterolemia and hypogonadism, were not normalized by INT-747 dosing into diabetic rats [83]. These findings suggest that INT-747 may improve *in vivo* penile erection affected by diabetes independently of the effects of this FXR agonist on glycemic control. However, additional mechanistic investigations are required to clarify the role of FXR signaling in erectile function.

INT-747 shows a modest agonistic activity for the G protein-coupled receptor TGR5 [86,87], increasing intracellular cAMP with an EC₅₀ of 20 μ M [71]. An increase in intracellular cAMP levels could lead to corpora smooth muscle relaxation and penile erection [88], therefore future studies are required to clarify a possible TGR5-mediated action of INT-747 in penile tissue. However, bile acid-induced vasorelaxation was not dependent upon stimulation of a bile acid surface membrane receptor, suggesting lack of TGR5 involvement [89]. In addition, the induction of eNOS and DDAH genes, both containing FXR responsive elements in their promoter region, by INT-747 [83] may indicate a prominent role of FXR activation in the INT-747-induced effects on erectile function.

6. Concluding remarks

Activation of FXR is involved in maintaining not only bile acid homeostasis, but also in regulating the intricate network governing

lipid and glucose homeostasis, highlighting potential beneficial effects in the treatment of MetS, a condition frequently associated with ED. The data reviewed here demonstrate that ED associated to metabolic derangements may be improved by FXR activation through molecular mechanisms which, although not yet fully elucidated, appear to be able to interfere with the RhoA/ROCK signaling and restore NO-mediated relaxation. FXR activation by chronic dosing with the selective FXR agonist 6 α -ethyl chenodeoxycholic acid (INT-747) improves erectile function in two different animal models of metabolic derangement by decreasing RhoA/ROCK signaling and restoring sensitivity to Ach and NANC stimulation. These results extend to the corpora cavernosa the protective activities mediated by FXR on the vasculature, revealing novel beneficial effects of INT-747 with potential clinical relevance. ED is now considered one of the first manifestations of MetS and a sentinel of forthcoming cardiovascular and metabolic events. FXR agonists, with the capacity to improve not only the metabolic profile but also erectile function, may offer to ED subjects with underlying conditions such as MetS, the chance to restore not only sexual but, most importantly, overall health.

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