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Nonalcoholic steatohepatitis as a novel player in metabolic syndrome-induced erectile dysfunction: An experimental study in the rabbit

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ABSTRACT

A pathogenic link between erectile dysfunction (ED) and metabolic syndrome (MetS) is now well established. Nonalcoholic steatohepatitis (NASH), the hepatic hallmark of MetS, is regarded as an active player in the pathogenesis of MetS-associated cardiovascular disease (CVD). This study was aimed at evaluating the relationship between MetS-induced NASH and penile dysfunction. We used a non-genomic, high fat diet (HFD)-induced, rabbit model of MetS, and treated HFD rabbits with testosterone (T), with the selective farnesoid X receptor (FXR) agonist obeticholic acid (OCA), or with the anti-TNFα mAb infliximab. Rabbits fed a regular diet were used as controls. Liver histomorphological and gene expression analysis demonstrated NASH in HFD rabbits. Several genes related to inflammation (including TNFα), activation of stellate cells, fibrosis, and lipid metabolism parameters were negatively associated to maximal acetylcholine (Ach)-induced relaxation in penis. When all these putative liver determinants of penile Ach responsiveness were tested as covariates in a multivariate model, only the association between hepatic TNFα expression and Ach response was confirmed. Accordingly, circulating levels of TNFα were increased 15-fold in HFD rabbits. T and OCA dosing in HFD rabbits both reduced TNFα liver expression and plasma levels, with a parallel increase of penile eNOS expression and responsiveness to Ach. Also neutralization of TNFα with infliximab treatment fully normalized HFD-induced hypo-responsiveness to Ach, as well as responsiveness to vardenafil, a phosphodiesterase type 5 inhibitor. Thus, MetS-induced NASH in HFD rab-

Abbreviations: ED, erectile dysfunction; MetS, metabolic syndrome; NASH, nonalcoholic steatohepatitis; CVD, cardiovascular disease; T, testosterone; FXR, farnesoid X receptor; OCA, obeticholic acid; RD, regular diet; Ach, acetylcholine; T2DM, type 2 diabetes mellitus; NAFLD, nonalcoholic fatty liver disease; PDU, penile doppler ultrasound; LUT, lower urinary tract; PAS, periodic acid-Schiff; PPARα, peroxisome proliferator-activated receptor alpha; PPARγ, peroxisome proliferator-activated receptor gamma; PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphatase; IL-12, interleukin-12; RORγt, retinoic-acid-receptor-related orphan receptor γt; ET-1, endothelin-1; ETA, endothelin receptor A; ETB, endothelin receptor B; αSMA, alpha smooth muscle actin; ROCK1, ROCK2, Rho-associated protein kinase type 1 and 2; RhoA, Ras homolog gene family, member A; TGFβ, transforming growth factor β; TIMP1 and TIMP2, tissue inhibitor of metalloproteinases-1 and -2; MMP2, MMP9, metalloproteinases-2 and -9; Cyp7A1, cholesterol 7 alpha-hydroxylase; SREBP1, sterol regulatory element-binding protein 1; VAMP4, vesicle-associated membrane protein 4; BSEP, bile salt export pump; SHP, small heterodimer partner; PLPA2, phospholipase A2; FN1, fibronectin 1; ADPN, adiponectin; AR, androgen receptor; IL-6, IL-8, IL-10, interleukin 6, interleukin 8, interleukin 10; MCP-1, monocyte chemoattractant protein-1, COX-2, inducible cyclooxygenase; CD68, macrophage marker; TLR2, TLR4, toll-like receptor 2 and 4; TNFα, tumor necrosis factor α; IL1β, interleukin-1β; GATA3, GATA-binding protein 3; AUC, area under the curve; eNOS, endothelial nitric oxide synthase; nNOS, neuronal nitric oxide synthase; VAT, visceral adipose tissue; TNFR1, TNFα receptor; CC, corpora cavernosa; PDE5, phosphodiesterase type 5; PKG1, protein kinase G 1; GCa1, GCb1, guanylate cyclase subunit a1 and b1.

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bits plays an active role in the pathogenesis of ED, likely through TNFα, as indicated by treatments reducing liver and circulating TNFα levels (T or OCA), or neutralizing TNFα action (infliximab), which significantly improve penile responsiveness to Ach in HFD rabbits.

1. Introduction

Metabolic syndrome (MetS) is a constellation of medical conditions, including centrally distributed obesity, decreased high-density lipoprotein cholesterol, elevated triglycerides, elevated blood pressure, and hyperglycaemia. MetS is recognized as a driver of the current epidemics of both type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD).

MetS, in the male, is also involved in the development of several non-metabolic diseases, including hypogonadism and erectile dysfunction (ED) (Corona et al., 2011a,b; Traish et al., 2011). A link between ED and MetS has also been envisaged, because both conditions are related to high odds of developing cardiovascular (CV) events (Corona et al., 2011b). In addition, in MetS patients, an important pathogenic component of ED is the associated hypogonadism (Corona et al., 2011a). Data from a consecutive series of more than 800 patients with sexual dysfunction indicate that patients with MetS have a component-dependent higher prevalence of ED and poorest penile Doppler ultrasound (PDU) parameters (Corona et al., 2006). However, no difference in terms of ED severity or PDU parameters was observed when MetS patients were stratified according to the presence of hypogonadism (Corona et al., 2006). Hence, the influence of factors other than hypogonadism, impairing hemodynamic mechanisms at both penile and systemic vascular bed levels, has been suggested.

Penile erection is the end result of a complex neurovascular process in which nerves, endothelium of sinusoids and blood vessels, and smooth muscle cells in the corpora cavernosa (CC) are involved. Indeed, it is well established that the balance between contractant and relaxant factors modulates the degree of smooth muscle tone of the CC and determines the functional state of the penis: flaccidity or erection. The most important and specific pathway for penile erection is the nonadrenergic/noncholinergic signaling, which through the release of a labile gas, nitric oxide (NO), leads to erection. Formation of NO is strictly controlled by the activity of NO synthase (NOS) isoenzymes: endothelial (eNOS) and neuronal (nNOS). NO activates the soluble guanylyl cyclase (GC), which increases 3',5'-cyclic guanosine monophosphate (cGMP) levels, thus regulating the activity of cGMP-dependent protein kinase (PKG) and calcium channels that affect the relaxation of CC smooth muscle. The main hydrolytic enzyme involved in cGMP breakdown, thus leading to penile flaccidity is the phosphodiesterase type 5 (PDE5). In human (Morelli et al., 2004) and rabbit (Morelli et al., 2013) penis expression of PDE5 is at least one-log unit higher than in other tissues. Impaired NO bioactivity is considered a major pathogenic mechanism leading to erectile dysfunction (Vignozzi et al., 2005).

Nonalcoholic fatty liver disease (NAFLD) is considered as the hepatic hallmark of MetS (Marchesini et al., 2003). The term NAFLD covers a spectrum of histological findings ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), the most severe form of NAFLD. Epidemiological studies indicate that NASH patients are at higher risk for CVD, independently from underlying cardiometabolic risk factors (Targher et al., 2008). In patients with NAFLD, the severity of liver injury and inflammation is strongly associated with increased CV and atherogenic risk (Alkhouri et al., 2010). This suggests that NAFLD is not merely a marker of MetS, but may also actively contribute to the pathogenesis of MetS-associated CVD, most probably through the release of pro-atherogenic inflammatory factors. This concept could be inferred to link NAFLD to ED.

The current study addressed this issue by taking advantage of a non-genomic, high fat diet (HFD)-induced, animal model of MetS that closely resembles the human MetS phenotype (Filippi et al., 2009; Vignozzi et al., 2011, 2012a; Maneschi et al., 2012; Maneschi et al., 2013). This model is characterized by hyperglycaemia, glucose intolerance, hypercholesterolemia, hypertriglyceridemia, hypertension, increased visceral fat mass, hypogonadotropic hypogonadism, lower urinary tract (LUT) abnormalities (Vignozzi et al., 2012a), penile alterations (Filippi et al., 2009; Vignozzi et al., 2011) and NASH (Maneschi et al., 2013). The primary goal of our analysis was to evaluate the relationship between liver pathology and penile dysfunction in the course of HFD-induced MetS. The data indicate that MetS-induced NASH plays an active role in the pathogenesis of ED in HFD rabbits, likely via TNFα. We have recently demonstrated that both testosterone (T; Filippi et al., 2009; Maneschi et al., 2012) or obeticholic acid (OCA; Vignozzi et al., 2011; Maneschi et al., 2013) supplementation to HFD rabbits were able to normalize not only several MetS features, including visceral adipose tissue dysfunction and insulin resistance, but also HFD-induced penile alterations, including hypo-responsiveness to acetylcholine (Ach). Hence, here, we tested whether these treatments can ameliorate also HFD-induced liver alterations, as well as TNFα circulating level. To test the effect of TNFα neutralization, a subgroup of HFD animals has been treated with the selective anti-TNFα mAb, infliximab. Interestingly, treatments which reduce liver and circulating TNFα levels, such as T and the FXR agonist OCA, or which neutralize TNFα action such as infliximab, significantly improve penile responsiveness to acetylcholine (Ach) in this model.

2. Material and methods

2.1. Chemicals

Phenylephrine (Phe) HCl, sodium nitroprusside (SNP), acetylcholine (Ach), were purchased from Sigma–Aldrich (St. Louis, MO, USA). Testosterone (T) supplementation was performed using T enanthate (250 mg; supplied by Bayer-Schering Pharma, Berlin, Germany). Obeticholic acid (OCA), a farnesoid-X receptor agonist, was supplied by Intercept Pharmaceuticals (New York, USA). Vardenafil, a selective phosphodiesterase type 5 inhibitor (PDE5i) was supplied by Bayer Schering Pharma AG, Global Drug Discovery (Wuppertal, Germany). Infliximab, an anti-TNFα chimeric mAb (Remicade, 100 mg, was from Janssen Biologics B.V. Einsteinweg 101 2333 CB Leiden, The Netherlands).

2.2. Animal model

The MetS rabbit model has been obtained by feeding adult male rabbits a high fat diet (HFD; $n = 48$) for 12 weeks, as previously described (Filippi et al., 2009). A first subgroup of HFD rabbits ($n = 28$) was treated with intramuscular injections of testosterone (30 mg/kg/week), as previously described (Filippi et al., 2009; Vignozzi et al., 2012a). A second subset of HFD rabbits ($n = 18$) was treated