Corpus Cavernosal Smooth Muscle Relaxation Effect of a Novel AMPK Activator, Beta-Lapachone

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ABSTRACT

Introduction. Adenosine monophosphate-activated protein kinase (AMPK) activation is suggested to relax smooth muscle by endothelial nitric oxide synthase (eNOS) phosphorylation.

Aim. To assess the mechanism and effect of a novel AMPK activator, beta-lapachone, upon cavernosal smooth muscle relaxation and the therapeutic potential for erectile dysfunction.

Methods. Human umbilical vein endothelial cells (HUVECs) were treated with beta-lapachone. The lysates were blotted with specific antibodies for phosphorylated AMPK (p-AMPK) or phosphorylated eNOS (p-eNOS). The membranes were re-blotted for total AMPK, total eNOS, or beta-actin. The eNOS activity was measured by the conversion of L-14C-arginine to L-14C-citrulline in HUVECs lysates. In a separated experiment, cavernosal strips from New Zealand white rabbits were harvested for organ bath study and the relaxation effect of beta-lapachone on phenylephrine-induced contracted strips was evaluated and compared with sodium nitroprusside, zaprinast, metformin, and aminoimidazole carboxamide ribonucleotide (AICAR). Methylene blue and L-NAME were used to assess the inhibition of cyclic guanosine monophosphate/nitric oxide pathway. Zinc-protoporphyrin-IX (ZnPP) was also used to investigate the contribution of mevalonate pathway.

Main Outcome Measures. The expression of p-AMPK, p-eNOS, AMPK and eNOS induced by beta-lapachone in HUVECs study and the percent relaxation of cavernosal tissue in organ bath study.

Results. Beta-lapachone clearly induced AMPK phosphorylation and, as a consequence, eNOS phosphorylation in HUVECs. Beta-lapachone-induced upregulation of eNOS activity was also observed in HUVECs and steadily increased up to 1 hour. In organ bath study, beta-lapachone significantly relaxed the phenylephrine pretreated strips in a dose-dependent manner. This relaxation effect was not totally blocked by methylene blue or L-NAME. After removing endothelium, the relaxation was totally blocked by ZnPP.

Conclusions. A novel AMPK activator, beta-lapachone has a strong relaxation effect on precontracted cavernosal smooth muscle strips in the rabbit. And phosphorylation of AMPK and eNOS strongly related to the action of beta-lapachone. Mevalonate pathway also might be considered as a suggestive mechanism. **Bae JH, Kim JW, Kweon GR, Park MG, Jeong K-H, Kim JJ, and Moon DG. Corpus cavernosal smooth muscle relaxation effect of a novel AMPK activator, beta-lapachone. J Sex Med 2011;8:2205–2214.**

Key Words. AMPK Activator; Beta-Lapachone; Penile Erection; eNOS; Corpus Cavernosum; Smooth Muscle Relaxation

Introduction

The evidence presented in the literature strongly suggests a link between obesity and erectile dysfunction [1]. This link is supported by the adverse effects of obesity on endothelial function, circulating androgen levels, an increased risk of diabetes, and hyperlipidemia, which comprise risk factors for erectile dysfunction [2,3]. Adenosine monophosphate (AMP)-activated protein

kinase (AMPK) is a key player in regulating energy metabolism, placing it at the center stage in studies of diabetes and related metabolic diseases [4]. Recent data have demonstrated that the enzyme plays a critical role in systemic energy balance. AMPK integrates nutritional and hormonal signals in peripheral tissues and the hypothalamus [5]. For example, adipose tissue acts as an endocrine organ that secretes a large number of hormones and cytokines that have systemic effects on processes such as glucose and lipid homeostasis, body weight regulation, blood pressure, and immune function [6]. Activation of AMPK suppresses 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which acts as a rate-limiting enzyme for endogenous cholesterol synthesis and is considered as a key enzyme in the mevalonate pathway or HMG-CoA reductase pathway [7,8]. The importance of interfering in the mevalonate pathway has been highlighted by numerous studies aimed at determining the cellular mechanisms underlying the pleiotropic effects of HMG-CoA reductase inhibitors (statins) [9]. Consistent with the known effects of statin treatment, the activation of the AMPK, either by the overexpression of a constitutively active AMPK mutant or by exposing endothelial cells to fluid shear stress, regulates HMG-CoA reductase activity and Ras-dependent signaling in endothelial cells via short- and long-term mechanisms. These involve the phosphorylation of the reductase by AMPK, the AMPK-dependent phosphorylation and degradation of forkhead transcription factor 1a, and decrease in HMG-CoA reductase expression, respectively [10].

From a large number of recent studies, it also seems that there is an intricate balance between the AMPK and the enzymes and signaling pathways that determine endothelial redox balance. For example, the AMPK can inhibit the formation of reactive oxygen species by the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and stimulate nitric oxide (NO) production by endothelial nitric oxide synthase (eNOS) [9,11]. There have been numerous reports of the AMPK-dependent phosphorylation of eNOS following endothelial cell stimulation with agents such as aminoimidazole carboxamide ribonucleotide (AICAR) and metformin [12,13]. This leads us to consider AMPK to induce eNOS activation and, possibly, cavernous smooth muscle relaxation.

Beta-lapachone (3,4-dihydro-2,2-dimethyl-2H-naphtho[1.2-b]pyran-5,6-dione) is a potent antitumor agent that stimulates NADPH : quinine oxidoreductase 1 (NQO1) activity. Beta-lapachone is reported to dose-dependently inhibit neointimal formation induced by balloon injury in the rat carotid artery model. In rat and human vascular smooth muscle cells, beta-lapachone increased the phosphorylation of AMPK [14].

We hypothesized that beta-lapachone would activate the phosphorylation of AMPK and eNOS. Therefore, it may have relaxation effect on the corpus cavernosal tissue. The aim of our study is to identify the effect of beta-lapachone on the AMPK and eNOS activation, and also to evaluate the relaxation effect on the cavernosum of the penis.

Materials and Methods

Human Umbilical Vein Endothelial Cells Study

Phosphorylation of AMPK and eNOS After Beta-lapachone Treatment

Human umbilical vein endothelial cells (HUVECs) were maintained in an endothelial growth media set (EGM-2 BulletKit; Lonza, Walkersville, MD). Cells were pretreated with 250 μM palmitate and 0.5% bovine serum albumin (BSA) in RPMI 1640 media for 1 hour and treated with 5 μM beta-lapachone for up to 2 hours. After treatment, adherent HUVECs were washed twice with phosphate-buffered saline, gently scraped from dishes, centrifuged, lysed in ice-cold lysis buffer that contained 50 mM Tris (pH 7.4), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mM ethylene glycol tetraacetic acid, 1 mM Na_3VO_4, and protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN, USA), and cleared by centrifugation. Equal amount (20 μg) of protein samples were subjected to electrophoresis on 10% polyacrylamide gels containing sodium dodecyl sulfate under reducing conditions. Separated proteins were electroblotted onto polyvinylidene fluoride membranes, and blots were incubated with 5% (w/v) nonfat dry milk for 1 hour and then washed with Tris-buffered saline containing 0.1% Tween-20. Primary antibodies against eNOS (Santa Cruz Biotechnology, Santa Cruz, CA, USA), phosphorylated eNOS (p-eNOS, Ser-1177, Cell Signaling Technology, Danvers, MA, USA), AMPKμ, phosphorylated AMPKα (p-AMPKα, Thr-172, Cell Signaling Technology), and β-actin (Santa Cruz Biotechnology) were diluted in 5% nonfat dry milk containing Tris-buffered saline with 0.1% Tween-20. Membranes were then incubated with primary antibodies, and antibody binding to the relevant proteins was detected using the appropriate secondary antibodies coupled with horseradish