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Characterization of TPN729 metabolites in humans using ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry

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ABSTRACT

TPN729 has been reported as a novel phosphodiesterase type 5 (PDE5) inhibitor to treat erectile dysfunction, and is currently being tested in clinical trials. In addition to the potent inhibition against PDE5, TPN729 is regarded as a better alternative to provide fewer side effects and better patient compliance. Given the potential therapeutic benefits of TPN729, it is of great importance to elucidate its metabolic characteristics in drug development. This study is the first to investigate the metabolic fate of TPN729 in humans. A rapid and reliable analytical method based on ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC/Q–TOF MS) was established to investigate the metabolic profiles of TPN729 in human plasma, urine, and feces after its oral administration. As a result, a total of 22 metabolites were identified, of which seven were confirmed in comparison with the reference substances. The incubations of the metabolite references in human hepatocytes and aldehyde trapping experiment were further conducted to investigate its metabolic pathways. The results of the present study indicated the extensive metabolism of TPN729 in humans, including oxidative deamination, oxidative ring opening, *N*-dealkylation, *N*-oxidation, hydroxylation, dehydrogenation, lactam formation, and glucuronidation. M3 resulting from *N*-dealkylation was the major circulating substance detected in human plasma. The principal metabolites detected in human feces were products of oxidative deamination and oxidative ring opening. The parent drug was identified as the major component in urine. Taken together, this study provided valuable information on the metabolic fate of TPN729 in humans, and applicable analytical strategies for rapid metabolic elucidation in complex matrix samples through the useful and reliable UPLC/Q–TOF MS technique.

1. Introduction

TPN729 (Fig. 1) has been reported as a novel phosphodiesterase type 5 (PDE5) inhibitor to treat erectile dysfunction (ED) [1], and is presently undergoing clinical trials in China. Preliminary pharmacokinetics of TPN729 in animals and humans has recently been reported [2]. As a potent inhibitor for PDE5 with an IC_{50} of 2.28 nM, TPN729 exerts its biological effects by selectively inhibiting PDE5 and blocking the degradation of cyclic guanosine monophosphate [1].

Currently available PDE5 inhibitors include sildenafil, vardenafil, and tadalafil (Fig. 1). These drugs exhibit potent inhibition of PDE5, thereby generating desirable pharmacological effects to the patients who suffer from ED [3]. However, the patients have to meanwhile endure adverse effects because of the less agreeable inhibitory selectivity profiles of these drugs. Sildenafil and vardenafil could cause visual disturbances due to the weak inhibition against PDE6 [4]; tadalafil inhibits PDE11, potentially causing backache and myalgia [5,6]. Given that diverse PDEs are expressed in various tissues, close attention should be paid to improving selectivity when developing new PDE5 inhibitors [5].

TPN729 is proved to be an effective PDE5 inhibitor and regarded as a better alternative with a selectivity profile of 2.5 times higher than sildenafil against PDE6 and 500 times higher than tadalafil against PDE11. Much better selectivity for PDE5 than the other PDE isozymes spotlights TPN729 a promising PDE5 inhibitor providing fewer side effects and better compliance. Moreover, TPN729 offers a longer duration of action than sildenafil in animal models, which reliably strengthens its therapeutic application [1]. Considering the potential therapeutic value and promising future use of

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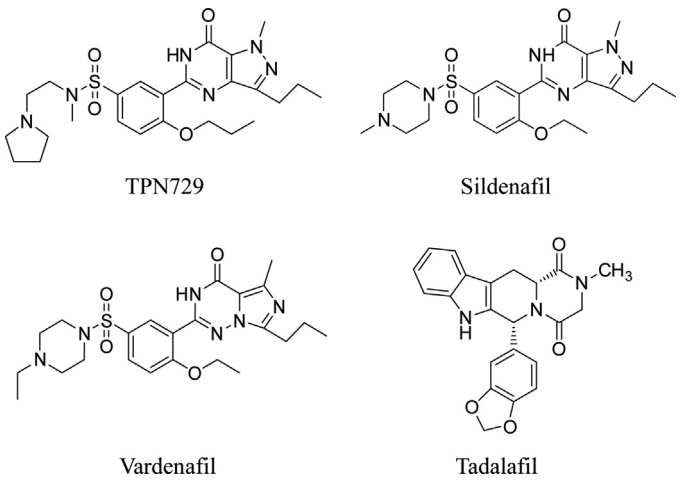

Fig. 1. Structures of TPN729, sildenafil, vardenafil, and tadalafil.

TPN729, its metabolic profiles in humans should be investigated at early development stage for safety and efficacy evaluation. However, to our knowledge, currently no published data concerning the metabolism of TPN729 in humans exist.

High-resolution mass spectrometry (HR-MS) has recently been employed as a reliable and powerful analytical technique for metabolic elucidation in biological matrices. The accurate mass measurements and valuable fragmentation information provided by HR-MS significantly contribute to the metabolite characterization [7–13]. In the present study, ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC/Q–TOF MS) was employed to investigate the metabolism of TPN729 in healthy humans. The metabolic profiles of TPN729 in human plasma, urine, and feces were elucidated for the first time. To identify the TPN729 metabolites, seven reference compounds were synthesized. Hepatocytes incubations of the metabolite references and aldehyde trapping experiment were conducted to further clarify its metabolic pathways.

2. Experimental

2.1. Chemicals and reagents

TPN729 maleate (purity 99.92%) and the reference substances of M11-1, M12-2, and M12-3 were kindly supplied by Topharman Shanghai Co., Ltd. (Shanghai, China). The reference compounds of M3, M8, M11-2, and M13 were prepared in our laboratory. Cryopreserved human hepatocytes were purchased from Xenotech, LLC (Lenexa, KS, USA). Pooled human liver S9 fraction was purchased from BD Gentest (Woburn, MA, USA). Williams' Medium E (WME) was purchased from Gibco Life Technologies (Grand Island, NY, USA). β-Glucuronidase (type H-3 from *Helix pomatia*, 90,000 units/mL), high-performance liquid chromatography (HPLC)-grade acetonitrile, methanol, methoxylamine, and formic acid were purchased from Sigma (St. Louis, MO, USA). HPLC-grade water was obtained from a Milli-Q gradient water purification system (Millipore, Molsheim, France). Potassium permanganate ($KMnO_4$) and other commercially available reagents were of analytical grade.

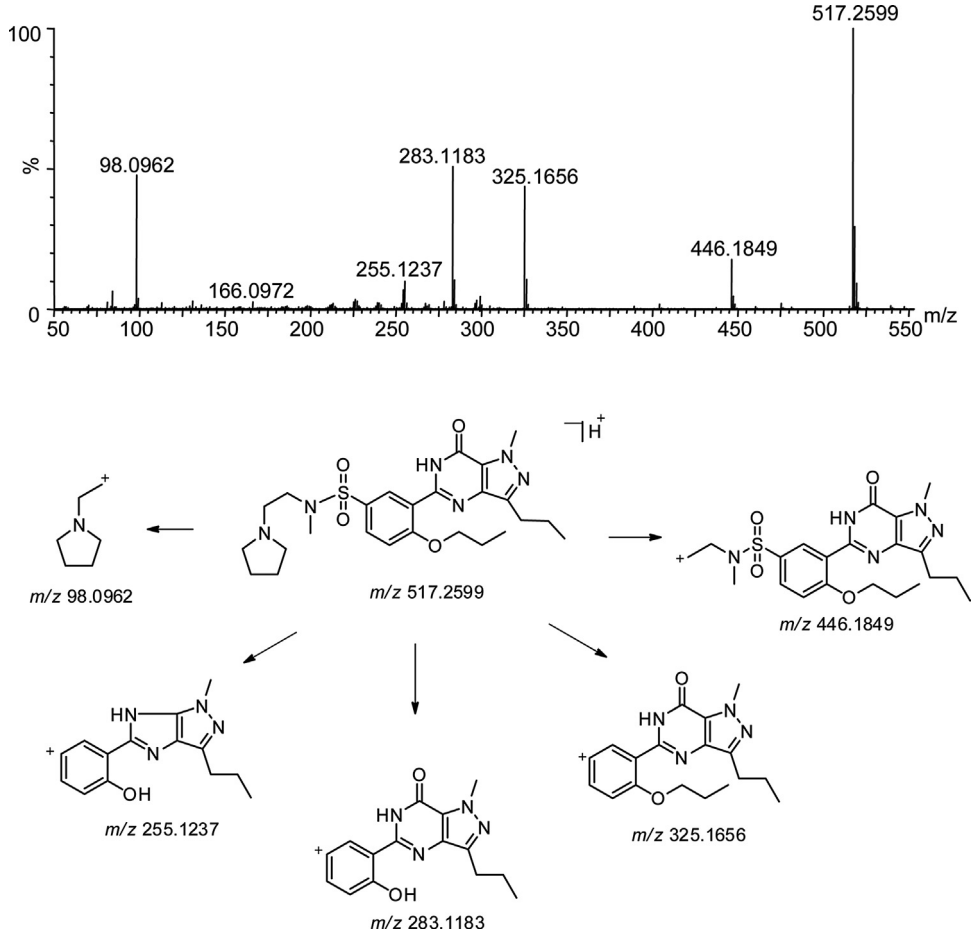

Fig. 2. MS^2 spectrum of TPN729 and its proposed fragmentation pathways.