ORIGINAL ARTICLE

Veterinary Research Forum. 2020; 11 (3) 213 – 217
doi: 10.30466/vrf.2019.95959.2302
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Metabolomic profiling of liver tissues after acute administration of vardenafil in rats

Onur Şenol[1]*, Şaziye Sezin Palabiyik[2], Beyzagül Polat[3], Yücel Kadioğlu[1], Mehmet Emrah Yaman[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy, Atatürk University, Erzurum, Turkey; [2] Department of Toxicology, Faculty of Pharmacy, Atatürk University, Erzurum, Turkey; [3] Department of Pharmacology, Faculty of Pharmacy, Atatürk University, Erzurum, Turkey.

Article Info

Article history:

Received: 18 October 2018
Accepted: 19 January 2019
Available online: 15 September 2020

Keywords:

Erectile dysfunction
Kreb's cycle
Metabolomics
Q-TOF MS
Vardenafil

Abstract

Erectile dysfunction (ED) diseases have almost affected 100 million men all over the world. Orally administered phosphodiesterase 5 (PDE 5) inhibitors are the most used pharmaceutical formulations for the treatment of ED. In this study, it is aimed to investigate the metabolomics feature of orally administered vardenafil in rats. To carry out the experimental procedure eight male Wistar albino rats were used. Their livers were gently removed and metabolomics profiles of each sample were determined by UPLC Q-TOF MS. Identification of metabolites was achieved by the METLIN database. Cluster analysis was also performed via Principle Component Analysis. Several metabolites were identified and results were evaluated by XCMS software. UPLC Q-TOF MS could be successfully applied to profile biomarkers and help us understand the molecular mechanisms of vardenafil usage. It was concluded that the level of some metabolites, responsible for the collagen synthesis and Kreb's cycle, has been statistically significant after the vardenafil administration.

Introduction

Erectile dysfunction (ED) is a widespread health problem in the general population of middle-aged men, defined as the lack of adequate penile erection to provide or continue for satisfactory sexual intercourse.[1-2] There are many different etiologies for the occurrence of ED including neurologic, hormonal, vascular pathologies, and also psychogenic factors. Another important finding of the cases of ED is that vast majority of them are closely related with various other problem commonly seen in men over 40 age such as prostate hypertrophy, arterial hypertension, ischemic heart disease, peripheral vascular disease, atherosclerosis, hyperlipidemia, and diabetes mellitus.[3]

Although there are several therapeutic methods, to improve erectile function including (sub-lingual, buccal, topical, etc.), oral treatments, in particular orally administered phosphodiesterase 5 (PDE 5) inhibitors are the first-line medication alternative for ED. Their favorable dose-response curve, tolerability of the medication, and short half-life by successfully demonstrating on-demand use make this pharmaceutical preferable.

The PDE 5 inhibitors (vardenafil, sildenafil, and tadalafil) are the most preferred pharmaceuticals for the treatment of ED.[4] Like the other PDE 5 inhibitors, vardenafil provides an increase in the intracellular cyclic guanosine monophosphate (cGMP) level due to inhibition of PDE 5 and eventually increases levels of nitric oxide (NO).[5] This strengthening effect allows control of erectile function to be restored without the presence of an unexpected response.[6]

Untargeted metabolomics studies are new strategies, known as top-down strategy, allow analysis of the global metabolomics profile without the need for a prior specific hypothesis on a large number of metabolites.[7]

In the presented study, it was aimed to detect as many groups of metabolites possible to obtain patterns or fingerprints associated with the effect mechanism of vardenafil using an untargeted metabolomics approach.

Materials and Methods

Chemicals and reagents. Vardenafil tablets (Levitra®; Bayer AG, Leverkusen, Germany) were purchased from a

***Correspondence:**

Onur Şenol. DVM, PhD
Department of Analytical Chemistry, Faculty of Pharmacy, Atatürk University, Erzurum, Turkey
E-mail: onursenol@atauni.edu.tr

local pharmacy store. Methanol, acetonitrile and formic acid were all high-performance liquid chromatography (HPLC) grade and obtained from Merck (Darmstadt, Germany).

Animal treatment and sample preparation. Male Wistar albino rats (200-250 g) were purchased from Ataturk University Experimental Animals Unit. All rats were kept in temperature-controlled cages (20.00 ± 4.00 ˚C), with a 12 hr light-dark cycle (lights on at 8:00) and a relative humidity of 50.00 ± 20.00%, with free access to food and water. Rats were randomly divided into two groups: A vardenafil-treated group (n= 4) and the control group (n=4). All animal experiments were conducted with the official approval of the Institutional Animal Care and Experiment Committee of Atatürk University (Approval No. 1600098868; 19.04.2016).

Vardenafil preparations were dissolved in water on the day of use and all rats were weighed before the administration. Vardenafil (10.00 mg kg^{-1}) was administered to each member of the vardenafil-treated group.[8] The rats in the vardenafil-treated group took a single oral dose of vardenafil. The rats in the control group received only water along with the experiment. All rats were kept in a cage for 8 hr after vardenafil administration and then gently euthanized with anesthetic ether. Liver tissues were removed, dissected, and washed in saline.

Liver tissues were ground in TissueLyzer II (Qiagen Inc., Rockford, USA). Amount of 100 mg was weighed in propylene tubes and 400 mL of the methanol-water mixture (3/1 v:v) were added for liquid-liquid extraction. Vortexing steps of 1 hr were accomplished via Eppendorf Thermomixer® equipment at 4.00 ˚C. Aliquots were centrifuged at 10,000 *g* for 10 min at 4.00 ˚C. Extracted samples were gently taken for Ultra Performance Liquid Chromatography Q-Time of Flight Mass Spectroscopy (UPLC Q-TOF MS) analysis. The mobile phase of the proposed method was a mixture of acetonitrile (ACN) (A)-water containing 0.10% formic acid (B) like the method of Wu *et al*.[9] Gradient elution were preferred as following procedure: The mobile phase A was 0.10% aqueous formic acid and mobile phase B was ACN. Gradient elution was started from 25.00% A for 5 min, then increased to 95.00% A in 25 min and then 5 min for reconstitution to a new sample. The total run time of the proposed method was 35 min. The flow rate was set to be 0.40 mL min^{-1}. Agilent ZORBAX Eclipse Plus (Agilent Technology Inc., Santa Clara, USA) C_{18}, (2.10 × 100 × 1.80 µm; column were used along with the analysis. The column temperature was 40.00 ˚C. Electron spray ionization was chosen in positive mode. 60-1000 m/z range were scanned for each analyte at 10.00 V of capillary voltage. The original chromatogram of the treated and control group was monitored in Figure 1.

Instrumentation. An Agilent 6530 UPLC Q-TOF MS (Agilent Technologies) coupled with Agilent 1290 Infinity LC system were used. The Q-TOF mass spectrometer system was operated in a positive capillary voltage of 3.50 kV in positive ion mode, drying gas flow of 11.00 L min^{-1}, and gas temperature of 350 ˚C. The nebulizer pressure was set at 45.00 psi. The fragmented voltage of the proposed method was set at 120 V. Accurate mass measurements along the analysis were received utilizing an automated calibrant delivery system using a jet stream thermal focusing ESI source that introduced a low flow (100 mL min^{-1}) of a calibrating solution (solution A; Agilent Technologies). The raw data were processed by Agilent MassHunter Software (version B.02.00; Agilent Technologies).

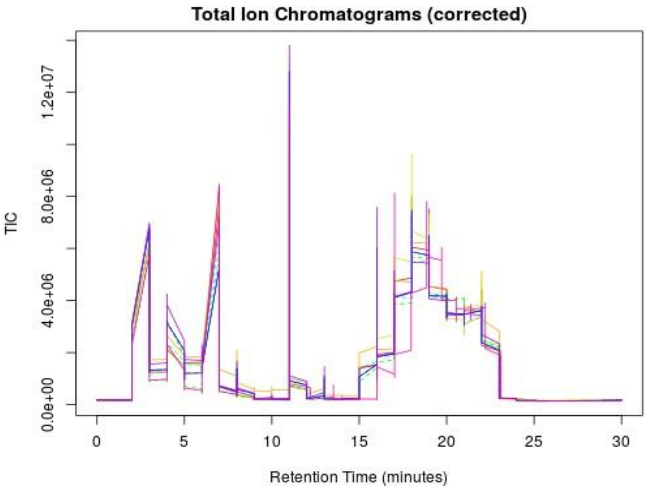

Fig. 1. Total ion chromatogram of vardenafil administrated and control group. Overlapped ion chromatogram of each sample including intensity versus retention time after peak correction.

Data processing and metabolite identification. 2500 different m/z values were identified by the MS detector which showed that 20,000 different parameters had to be evaluated to interpret what happened after vardenafil administration. Several variations could occur either within- or between-batch in signal intensity which led to noise in the data and due to that reason statistical analysis was badly effected to extract and identify interested metabolites.[10-11] For this reason, Wavelet transformation algorithms were improved for retention time and peak correction avoiding within and between batch noises. Peaks were normalized, aligned, and pre-processed by XCMS 2018 online version (xcmsonline.scripps.edu) by centWave algorithm. All retention time corrections were achieved by OBI-warp algorithm. Total ion chromatogram were monitored in Figure 2.

Results

The multivariate analysis method was used to evaluate UPLC Q-TOF MS data to make metabolite variations prominent and to identify the metabolites responsible for such variations. The corresponding score plots from the supervised Partial component analysis (PCA) showed a clear difference between the two groups (Fig. 3).

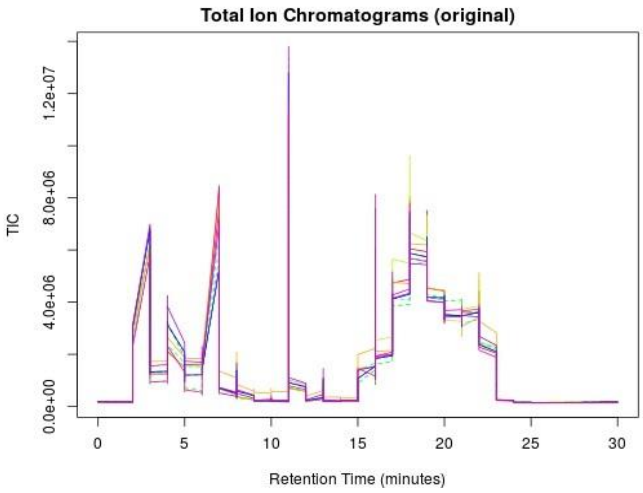

Fig. 2. Aligned ion chromatograms of each sample before the peak correction. Raw intensity vs retention time graph.

The cloud plot, which was derived from the fold analysis and *p*-value (Mann-Whitney unpaired) of each measurement was used to select potential biomarkers and visualize metabolomics changes in Figure 4.

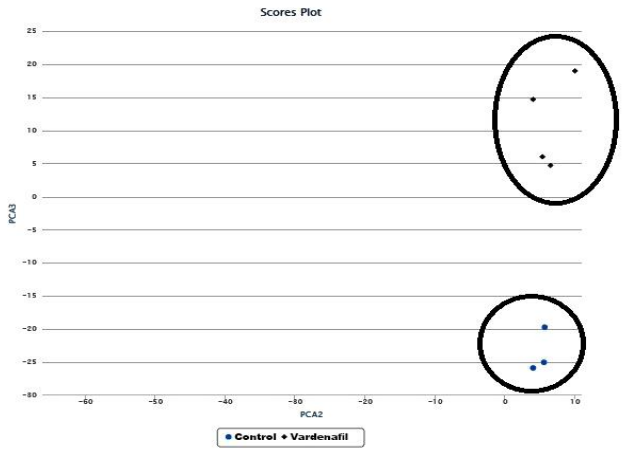

Fig. 3. Score plots for two principle components of multivariate data analysis model classifying drug administered group vs. controls.

The wider red/green dots were the most likely potential biomarkers because of their high contributions and correlations. A list of the identified biomarkers which were greater than 1.50 fold and 0.01 of *p*-value are given in Table 1. The intensities of the peaks obtained from the Agilent 6530 UPLC Q-TOF MS instrument and were used to calculate the relative concentrations of the biomarkers.

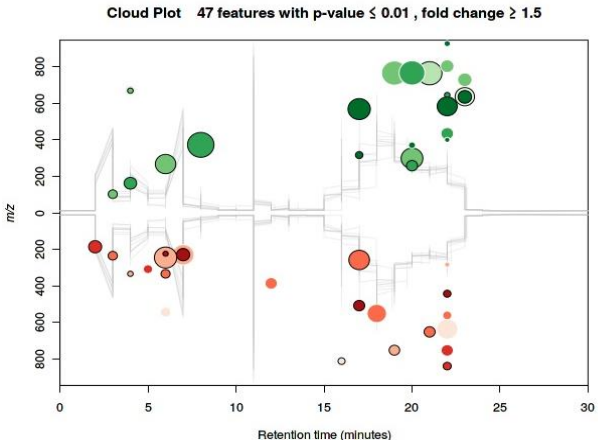

Fig. 4. Illustration of significant features between drugs administered group vs. controls that have ≤ 0.01 *p*-value and ≥ 1.5 fold between each group.

The main aim of any omics work is to perform a global snapshot of metabolites in any biological medium or other analytical situation without observational biases. Nevertheless, such high information content encountered in a challenge as forming biologically relevant conclusions from any dataset indeed requires advanced forms of data analysis. To achieve such purposes, metabolite identification is a crucial section in omics studies. To identify metabolites, mass to charge ratio, and retention time data were utilized. All data obtained, including blank, QC, and plasma samples were exported to Agilent MassHunter Software (version B.02.00), and then received results were transferred into XCMS software programs for peak correction and peak list alignment. In this study, almost 47 m/z ratios were determined by the detector.

Table 1. Identified compounds with fold analysis.

Compound	Fold	Retention time (min)	*p*-value
1,3-bis(2-ethylhexoxy)propan-2-amine	+ 2.30	17.00	0.0004
Isoleucyl-Proline	-12.60	7.00	0.0025
[2-(10-acetyloxy-3,7,9,11-tetramethyltridecyl)-5,6-dimethoxy-3-methylpyridin-4-yl] acetate	-6.10	17.00	-
Asp His Arg Arg	+355.40	22.00	0.0018
5-Oxo-L-proline	+14.60	23.00	-
Asp Ile Pro Val	-2.30	22.00	0.0045
1-Aminohexadecan-2-ol	+6.20	20.00	0.0032
N-Methylglutamic acid	+10.00	4.00	0.0021
L-Asparaginyl-L-tyrosyl-L-valyl-L-isoleucyl-L-histidine	+1.60	22.00	0.0044
Allysine	+3.40	3.00	0.0016
4-(Methanesulfinyl)-6-(methanesulfonyl)-2-methylpyrimidine	-3.50	3.00	0.0027
5-Pyrimidinecarbonitrile, 4-(methylthio)-2-phenyl-6-(2-phenylhydrazino)-	+1.60	4.00	0.0021
PC(14:0/20:5(5Z,8Z,11Z,14Z,17Z))[U]	-5.10	19.00	0.0039
PI(17:0/14:1(9Z))	-2.10	16.00	0.0027

Features that provide the following criteria: $p \leq 0.01$ and fold analysis > 1.5 from m/z scores were considered to be potential biomarker metabolites. It is observed that 47 m/z signal passed these criteria (Fig. 1). These features were taken into account for identification. Unfortunately, m/z scores of several features point at more than one metabolite. The debate in m/z score was overcome by checking MS/MS results of blank, QC samples.

Discussion

Some interesting results were obtained from the fold analysis. 5-oxo-proline and glutamic acid levels were strongly high in the vardenafil treated group. Glutamic acid is a non-essential amino acid that plays a role in the metabolic intermediate of Kreb's cycle. It also plays an important role in ammonia elimination.[12-13] Glutamic acid is derived from 5-oxo-proline via a 5-oxo-prolinase enzyme. An increase in the glutamic acid level may be caused by the increase in 5-oxo-proline. Change in these metabolites is thought that the energy metabolism of the rats was significantly differentiated. The biochemical mechanism of this cycle was monitored in Figure 5.[14] In addition to these isoleucyl proline level was also lowered by the fold analysis table. Isoleucyl proline plays a crucial role in collagen synthesis and alteration in its concentration inhibits the synthesis of this important protein. Deficiency in collagen cause rigidity in internal organs and leads to hazardous influences.

Allysine is another finding to comparative analysis. It is a lysine derivative and mostly contributes to collagen and elastin synthesis. Increase in allysine level indicates that collagen levels were lowered significantly and organism may try to stabilize the situation.

Phosphatidylcholine (14:0/20:5(5Z,8Z,11Z,14Z,17Z)) levels were also lowered in the vardenafil administered group. This is a fatty acid chain and its bonds were broken. Vardenafil may influence Kreb's cycle as mentioned above. Due to that reason, energy consumption is getting higher. Thus, fatty acid chains may be consumed because of that reason. In addition to this, it is reported that glycero phospholipids, PI(17:0/14:1(9Z)), were also lowered. Both findings revealed that energy demand increases with vardenafil administration and its affect lipid metabolism.

Pharmacometabolomics study, as a systemic approach, aims to provide information on the effect of vardenafil in liver tissue. Metabolite profiling can aid the mechanistic elucidation of toxicological and pathologic changes. It has been demonstrated that our metabolomics method based on UPLC Q-TOF MS could be successfully applied to plasma samples and help us understand the molecular mechanisms of vardenafil usage in rats. This study was the first metabolomics application after a single administration of vardenafil on male rats. The obtained results showed that vardenafil might affect Kreb's cycle and collagen synthesis pathways. Lipid metabolism and energy metabolism were significantly affected by vardenafil.

Acknowledgment

Thanks to the Scientific Research Fund of Ataturk University (Project No. 2016/117).

Conflict of interest

There is no conflict of interest to declare regarding the proposed study.

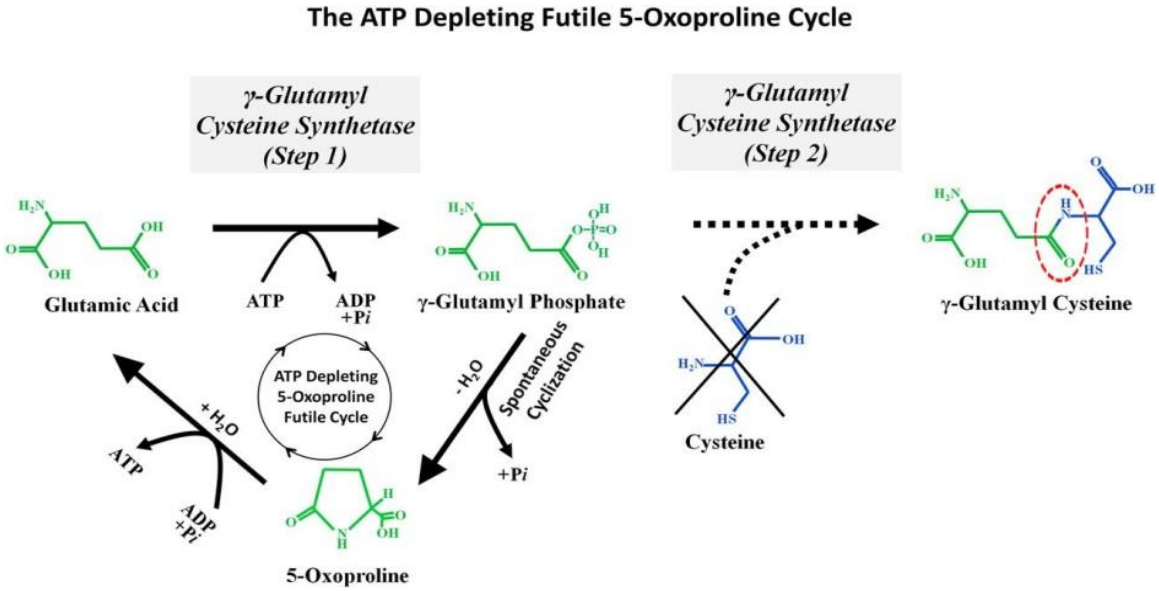

Fig. 5. 5-Oxo proline pathway which found statistically important by feature annotation.

References

1. Hellstrom WJG, Gittelman M, Karlin G, et al. Vardenafil for treatment of men with erectile dysfunction: Efficacy and safety in a randomized, double-blind, placebo-controlled trial. J Androl 2002;23(6):763-771.
2. Fugl-Meyer AR, Lodnert G, Bränholm IB, et al. On life satisfaction in male erectile dysfunction. Int J Imot Res 1997;9(3):141-148.
3. Chew KK, Earle CM, Stuckey BG, et al. Erectile dysfunction in general medicine practice: Prevalence and clinical correlates. Int J Impot Res 2000; 12(1):41-45.
4. Thoma C. Erectile dysfunction: Vardenafil in men with T2DM. Nat Rev Urol 2016;13(3):126. doi: 10.1038/ nrurol.2016.30.
5. Montorsi F, Hellstrom WJG, Valiquette L, et al. Vardenafil provides reliable efficacy over time in men with erectile dysfunction. Urology 2004; 64(6):1187-1195.
6. Setter SM, Iltz JL, Fincham JE, et al. Phosphodiesterase 5 inhibitors for erectile dysfunction. Ann Pharmacother 2005;39(7-8):1286-1295.
7. Alonso A, Marsal S, Julià A. Analytical methods in untargeted metabolomics: State of the art in 2015. Front Bioeng Biotechnol 2015; Doi:10.3389/fbioe. 2015.00023.
8. Radovits T, Bömicke T, Kökény G, et al. The phosphodiesterase-5 inhibitor vardenafil improves cardiovascular dysfunction in experimental diabetes mellitus. BrJ Pharmacol 2009;156(6):909-919.
9. Wu Q, Zhang H, Dong X et al. UPLC-Q-TOF/MS based metabolomic profiling of serum and urine of hyperlipidemic rats induced by high fat diet. J Pharm Anal 2014;4(6):360-367.
10. Veselkov KA, Vingara LK, Masson P, et al. Optimized preprocessing of ultra-performance liquid chromate-graphy/mass spectrometry urinary metabolic profiles for improved information recovery. Anal Chem 2011;83(15):5864-5872.
11. Wang J, Li Z, Chen J, et al. Metabolomic identification of diagnostic plasma biomarkers in humans with chronic heart failure. Mol Biosyst 2013;9(11):2618-2626.
12. Kretovich WL, Kariakina TI, Kazakova OV, et al. Biosynthesis of amino acids from sucrose and Krebs cycle metabolites by Rhizobium lupini bacteroids. Mol Cell Biochem 1983;51(1):61-66.
13. Tapiero H, Mathé G, Couvreur P, et al. II. Glutamine and glutamate. Biomed Pharmacother 2002;56(9):446-457.
14. Fenves AZ, Kirkpatrick HM, Patel VV, et al. Increased anion gap metabolic acidosis as a result of 5-oxoproline (pyroglutamic acid): A role for acetaminophen. Clin J Am Soc Nephrol 2006;1(3):441-447.