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Article

Metabolomics approach: Interpretation of changes in rat plasma metabolites after solifenacin treatment

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Changes in metabolite levels of patients using the long-term drug can be comprehensively demonstrated by pharmacometabolomic studies. In this study, biological alterations induced by the administration of solifenacin succinate were investigated with a pharmacometabolomics approach on rat metabolism. Plasma samples obtained from rats were analyzed by LC-Q-TOF/MS/MS. METLIN and HMDB databases were used to identify metabolites. Data were processed and classified with MATLAB 2017b. 53 *m/z* values were found to be significantly different between the drug and control groups ($p \leq 0.01$ and fold analysis > 1.5) and identified by comparing METLIN and HMDB databases. According to multivariate data analysis, changes in arachidonic acid, thromboxane A2, palmitic acid, choline, calcitriol, histamine phosphate, retinyl ester, l-cysteine, l-leucine, beta-alanine, l-histidine levels were found to be statistically significant compare to the control group. Differences in the biosynthesis of phenylalanine, aminoacyl-tRNA, tyrosine, tryptophan, metabolism of glycerophospholipid, cysteine, methionine, histidine, arachidonic metabolism have been successfully demonstrated by the metabolomics approach. Our study provides important information to explain the efficacy and toxicity of chronic administration of solifenacin succinate.

Keywords: Solifenacin succinate. Metabolomics. Rat. LC-Q-TOF/MS/MS.

ABBREVIATIONS

OAB: Overactive bladder
GC: Gas chromatography
LC: Liquid chromatography
NMR: Nuclear magnetic resonance
MS: Mass spectrometry
IS: Internal standart
QC: Quality control
(HMDB): Human Metabolome Database
OPLS-DA: The orthogonal partial least squares discriminant analysis
RMSEC: The raw data with the lowest root mean square calibration error
AUC: The area under the curve
ROC: The receiver operator characteristic curve

INTRODUCTION

Overactive bladder (OAB) is a common and negative syndrome that significantly affects patients' quality of life. Symptoms observed in OAB, such as sudden urge to urinate, incontinence, frequent urination at night or day, are thought to be due to abnormalities in detrusor smooth muscle function and/or nervous system. After evaluating symptoms and lifestyle factors such as drug history, diet, and fluid intake, pharmacological treatment methods are prescribed (Basu, 2019). Antimuscarinic agents are mostly preferred in the pharmacological treatment of OAB. Muscarinic M_3 subtype receptor

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blocker solifenacin succinate; It inhibits frequent and uncontrolled contractions of detrusor and peripheral muscle fibers by inhibiting neuromuscular regions that provide bladder contraction through acetylcholine. Thus, mental, social, and physical limitations in incontinence, frequent urination, and the quality of life due to these symptoms are eliminated (Masunaga *et al.*, 2008). Solifenacin succinate is generally used for a long time until the symptoms disappear.

Metabolomics examines the identities, quantities, and changes of endogenous metabolites in biological systems after any attempt such as medication, dietary disease, surgical intervention and focuses on metabolic networks rather than a single metabolite (Ryan, Robards, 2006). Pharmacometabolomics; it aims to define the metabolic fingerprint associated with variations in drug response or toxicity to interpret inter-individual variations in the metabolism characteristics of the drug. This approach can provide metabolic signatures associated with drug side effects and predicted drug responses by analyzing differences in metabolic profiles. It can also provide deep insights into the complexity of drug response that may not be adequately understood by other means, such as genetic variations (Amin *et al.*, 2018).

Many metabolic reactions occur in the human body depending on the drug administration. Thus, changes in the concentration of certain metabolites may be observed in biological fluids due to drug administration (Shi, 2017). As a result of the development of analytical technologies in recent time, metabolites in biological fluids (urine, plasma, serum, saliva, and biopsy samples) of humans who have been administered and/or not administered the drug can be determined without focusing on a particular compound. Findings obtained by targeted metabolomics analysis using gas chromatography (GC), liquid chromatography (LC), or nuclear magnetic resonance (NMR) combined with various instrumental methods (mass spectrometry (MS) allow predictions of drug toxicity or efficacy. With the pharmacometabolomics approach, it is possible to have an idea about the possible side effects of drugs (Mamas *et al.*, 2011). Side effects such as constipation and dry mouth, dizziness, blurred vision, colon obstruction, intracranial bleeding, and gastrointestinal bleeding have been reported in many clinical studies on solifenacin succinate. However, the main theme of these studies has been the efficacy and safety of the drug. Side effects and toxicity due to long-term use of solifenacin succinate have not been studied in detail (Chapple *et al.*, 2004; Chu, Smith, Uchida, 2009; Benner *et al.*, 2010; Maman *et al.*, 2014; Drake *et al.*, 2016).

Plasma samples of the drug (solifenacin succinate administered) and the control groups were analyzed by LC-Q-TOF-MS/MS in the study. By comparing metabolome profiles of the groups, remarkable information was obtained in order to explain the drug exposure at the molecular level.

MATERIAL AND METHODS

Reagents and chemicals

The chemicals used in the study are as follows; deionized water (R> 18.2 M) cm-1; PURELAB Ultra system; Elga Labwater, Ede, The Netherlands), Formic acid (Riedel-de-Haën ™, Honeywell,), Acetonitrile, 98%, (Sigma-Aldrich, St. Louis, MO, USA). Olanzapine active substance was used as an internal standard. Materials such as 5 mL and 2 mL conical bottom screw cap tube (BD Biosciences Discovery Labware, CT, USA), single-use sterile pipettes (0.1, 1.5, 10 mL), 96-well cell culture plates (Eppendorf, UK), 1.5 mL disposable tube (Eppendorf, UK), 0.45 µm syringe filter tips (membrane) were used in this study.

Animal experiments

12 albino wistar rats weighing between 300 and 350 g were used in the study. The rats were kept in clear plastic cages on sawdust beds at 22 °C and in a well-ventilated room (humidity 60%) under sensitive light conditions (12/12 hours light/dark cycle) approved by the local animal care committee of Atatürk University (26.06.2015/122). It was studied in accordance with the national guidelines for the use and care of laboratory animals in the administration of drugs and anesthesia to the rats and the sacrifice of the rats. Approval certificate was obtained from The Local Animal Care Committee of Atatürk University for animal study (11.07.2017/75296309-

050.01.04- E.1700196080). The 10-month-old rats were randomly divided into two groups (n = 6) which are control and drug-administered groups. All rats have free access to pellet laboratory chow and water for 10 days from sampling to the morning of surgery. 1 mL of water containing 0.026 mg solifenacin succinate was given to the drug group at 10:00 a.m. by gavage. The same volume of saline solution (pH 7.4) was also given to the control group. 25 mg kg^{-1} of thiopental sodium was applied intraperitoneally to animals for anesthesia. After blood was drawn from the heart of the rats, the rats were sacrificed with high-concentration anesthesia. Blood samples were centrifuged at 1000 g for 5 minutes at +4 °C in EDTA tubes (Lu *et al.*, 2020). Plasma samples were extracted and stored at -80 °C until analysis.

Sample preparation for metabolomics study

Our extraction procedure is based on the previous work (Figure 1) (Gundogdu *et al.*, 2019; Senol *et al.*, 2019). All samples were randomized in order to get rid of batch drift. Samples were extracted and given to LC-Q-TOF/MS/MS system in a random order. After plasma samples were thawed at room temperature, they were homogenized on a vortex mixer. 60 µL of plasma samples were taken into Eppendorf tubes and 300 µL acetonitrile was added for extraction. The mixtures were vortexed for about 1 minute and incubated at +4 °C for 15 minutes. The mixture was centrifuged at 16000 rpm for 15 minutes at 4 °C. After centrifugation, the supernatants from the upper phase were transferred to a new tube and all of them were blown off in a vacuum concentrator in V-Aqua mode at low pressure. The residue was dissolved in a mixture of mobile phase including acetonitrile and 0.1% formic acid containing 50 ng/mL of olanzapine, (internal standard, IS).

The integration success of the XCMS software was evaluated by the internal standard. QC (Quality Control) samples and The Relative Standard Deviation (RSD%) values were used in the study to determine reproducibility. All features which have higher than 20% RSD values and could not be detected in QC analysis were removed from the peak table. The IS concentration in the mobile phase was 1 µM and IS is used to improve data quality and check the autonomous integration success of the software. The repeatability of the proposed method was checked by injecting QC after every 8 samples during the analytical run. For the preparation of QC samples, 5 µl of each sample was taken and collected in an Eppendorf tube. Thus, a QC pool was created and injected every 8 measurements along with the analysis (Kuligowski *et al.*, 2015).

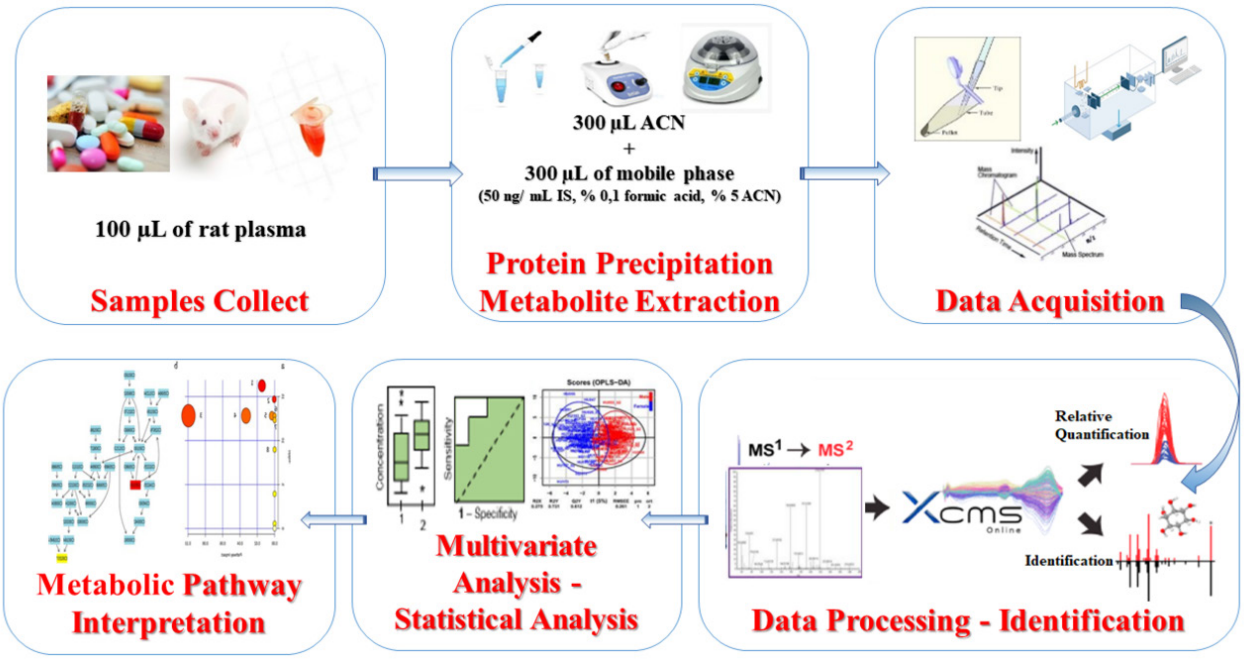

FIGURE 1 - Schematic representation of the metabolomic workflow.

Conditions of LC-Q-TOF/MS/MS

In this study, the Agilent 1290 Infinity LC system coupled with an Agilent 6530 True-Mass Q-TOF mass spectrometer (Agilent, USA) was used to elute and identify plasma metabolites. The Q-TOF mass spectrometer system was used in Dual Agilent Jet Stream Electrospray Ionization (AJS ESI) ion mode and optimized at 3.5 kV positive capillary voltage in positive ion mode. While the drying gas was flowing at 11 L/min, the temperature was kept at 350 °C. In the chromatographic separation, Hypersil C18 column (100 mm x 2.1 mm, 1.9 µm) was used. A mixture containing acetonitrile and ultra-purified water (0.1% formic acid) was used as the mobile phase. The mobile phase was run at a rate of 0.4 mL/min and in gradient elution mode. Run time was set as 15 minutes.

Data processing

All data from the blank, QCs, and plasma samples were transferred to Agilent Mass Hunter Software Version B.02.00. The peaks appearing on the chromatogram were deconvoluted by using the XCMS software program. A peak list of features was obtained, pre-processed and normalized.

In the proposed work, peak detection was performed via XCMS centWave algorithm. Peaks were aligned by the Obiwarp approach found in XCMS. Then peak grouping step was achieved to obtain unique retention time and *m/z* value which called feature for the analysis. A peak table including *m/z* results, retention time, and intensities of each feature were generated by the package. Data were transferred to MATLAB for pre-processing and chemometric analysis.

Features that have low reproducibility in QCs and detected in blanks were removed. Missing values lower than 20% RSD were also removed. Column median imputation method was selected for filtering. No transformation was applied to the data. Normalization was performed by cyclic loess.

Multivariate data analysis for metabolite profile

Agilent Mass Hunter Software version B.02.00 (Agilent Technologies, USA) program was used to evaluate chromatogram from blank, QC, and plasma samples. The blank solution was used in order to see there exists any systematical ghost peak and impurity on the system. Also our mobile phase and internal standard were evaluated in terms of impurities and false peaks (Kuligowski *et al.*, 2015). To correct the peaks, chromatograms were transferred to XCMS software. Features with $p \leq 0.01$ and fold analysis > 1.5 in terms of *m/z* scores were considered to be statistically significant. The determined *m/z* scores were compared with the Human Metabolome Database (HMDB) and METLIN for identification. MS/MS spectra of each feature were also evaluated with the reference spectra of METLIN & (HMDB). Data collection from the identified metabolites and cluster analysis of drug & control groups were performed with the orthogonal partial least squares discriminant analysis (OPLS-DA) algorithm in the MATLAB R2017b program. The presence of metabolites that allowed the separation of the metabolite profiles of the drug and control groups was demonstrated by OPLS-DA analysis. Metabolic pathways in which metabolites are effective were determined with MetaboAnalyst 4.0 Software.

RESULTS

OPLS-DA Model

Scientific studies are too complex to be explained with a single variable. There may be more than one variable affecting the data obtained. Therefore, univariate data analysis is limited and not enough to explain the comprehensive effects of any exposure. So multivariate data analysis was remarkably important to explain the biochemical situation. Classification of the drug and control group were determined using OPLS-DA. For confident cluster analysis, data should be processed by removing the interference, false peak, and low-intensity signals. Intensity lower than 3000 were filtered. Interferences that were not found in QC samples were also filtered. Three latent variables were used to explain the OPLS-DA model. OPLS-DA score graphs consisting of 2 clusters belonging to groups were obtained. In OPLS-DA score graphs, the clustering of the samples belonging to the two groups show that there are significant differences

between the metabolic profiles of the drug and the control groups (Figure 2). The proposed model explained 96.13% of the raw data with the lowest root mean square calibration error (RMSEC). In addition, the predictability of the proposed model was evaluated by cross-validation. While RMSEC was 0.01, the regression coefficient was 0.99. While RMSEC was 0.012 for the data set and 0.024 for cross-validation, the regression coefficient was 0.991 and 0.972, respectively. The RMSEC value confirms that the data fit well with the proposed model. The fact that the R^2 Calibration value was close to 1 showed a very high correlation between variables and datasets. The stability and reliability of the model were acceptable. The sensitivity and selectivity of the model were determined by the area under the curve (AUC) value of the ROC (Receiver Operator Characteristic Curve) curves (Figure 3). The AUC being close to 1 indicates that the model's sensitivity and selectivity are good.

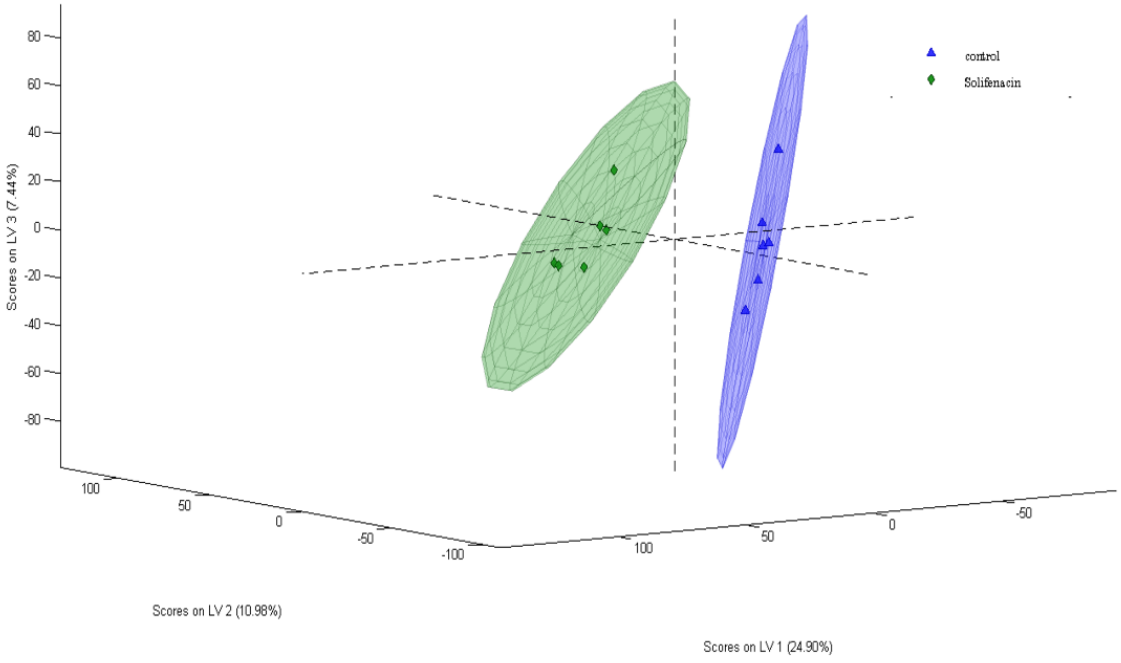

FIGURE 2 - OPLS-DA Score plot for drug-administered group (green) vs control group (blue).

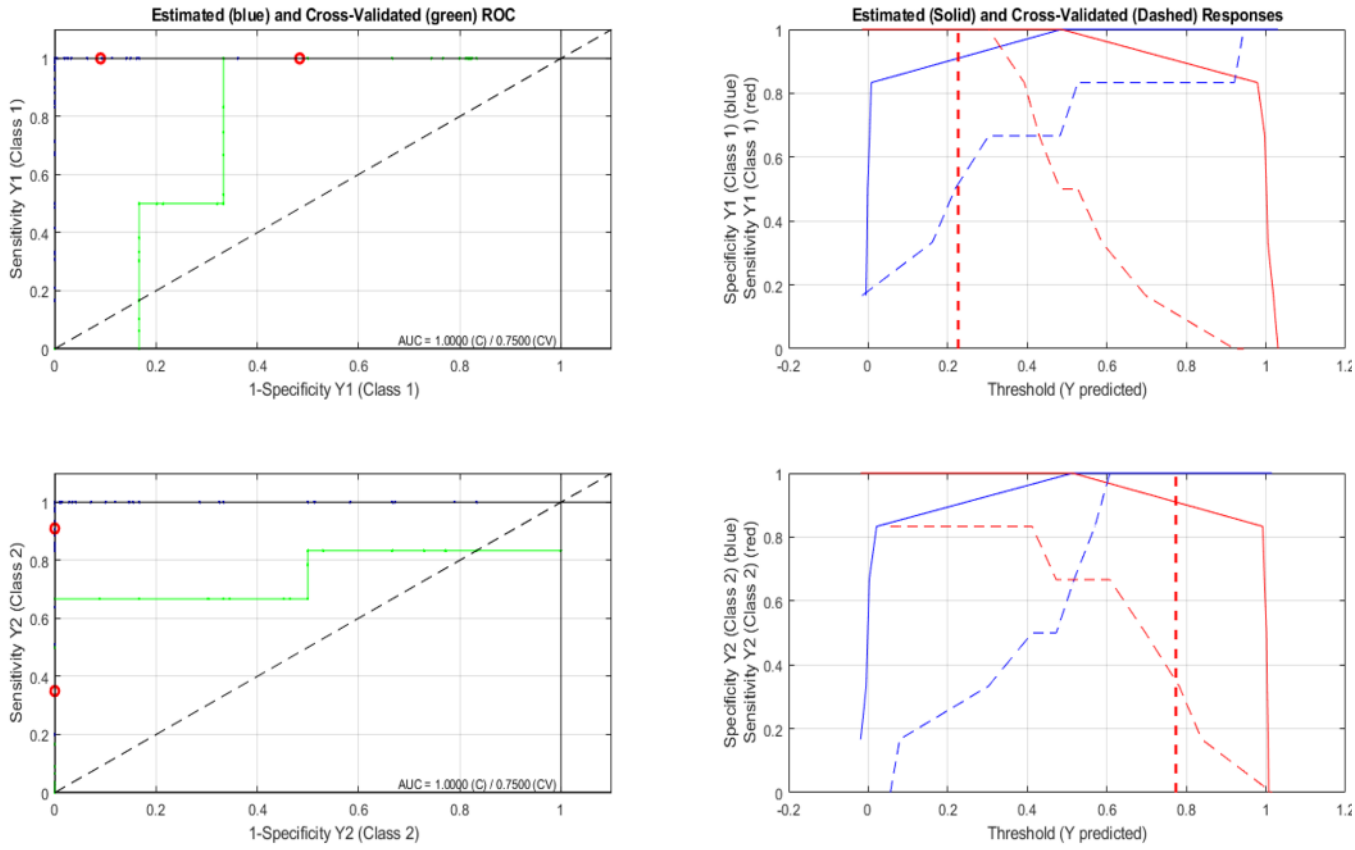

FIGURE 3 - Sensitivity and selectivity of models determined by the area under the curve of the ROC (Receiver Operator Characteristic Curve) curves (AUC).

Statistical analysis

Permutation test performed via 50 iterations. Self-prediction values in the permutation test were 0.018, 0.040, and 0.043 according to Wilcoxon, significant test, and random t-tests, respectively. Cross-validated values with permutation test were found as 0.026, 0.037, and 0.041 according to Wilcoxon, significant test, and random t-tests, respectively. The fact that our values are less than 0.05 shows that our proposed model according to the permutation test is important at the 95% confidence level.

Identification of metabolites

For the identification of metabolites, two criteria were used, which are the mass-to-charge ratio and retention time of the properties detected by LC-Q-TOF-MS/MS. Normalization was made to the MS/MS results of all samples. Normalized and non-normalized data using cyclic loess were monitored in Figure 4. While the detector detected 4440 different *m/z* scores for the drug group in the list, 53 different metabolites which differ significantly from the control group by having the fold analysis > 1.5 and $p \leq 0.01$ were found (Figure 5 and Table I). Although these features are taken into account for identification, *m/z* scores of various features may indicate more than one metabolite. This confusion due to *m/z* scores was eliminated by comparing MS/MS results.

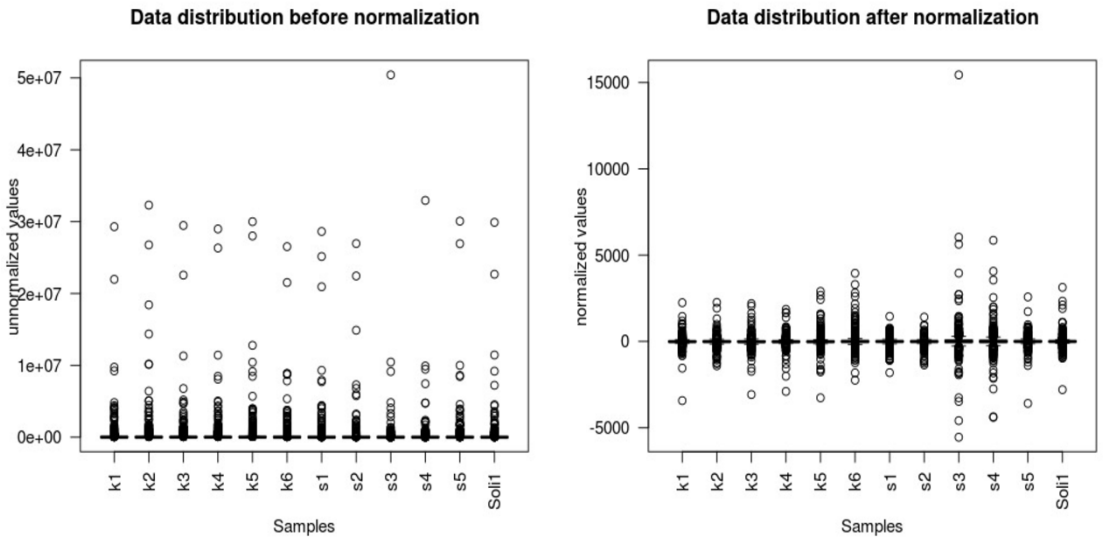

FIGURE 4 - Normalization of the TOF-MS data via cyclic Loess.

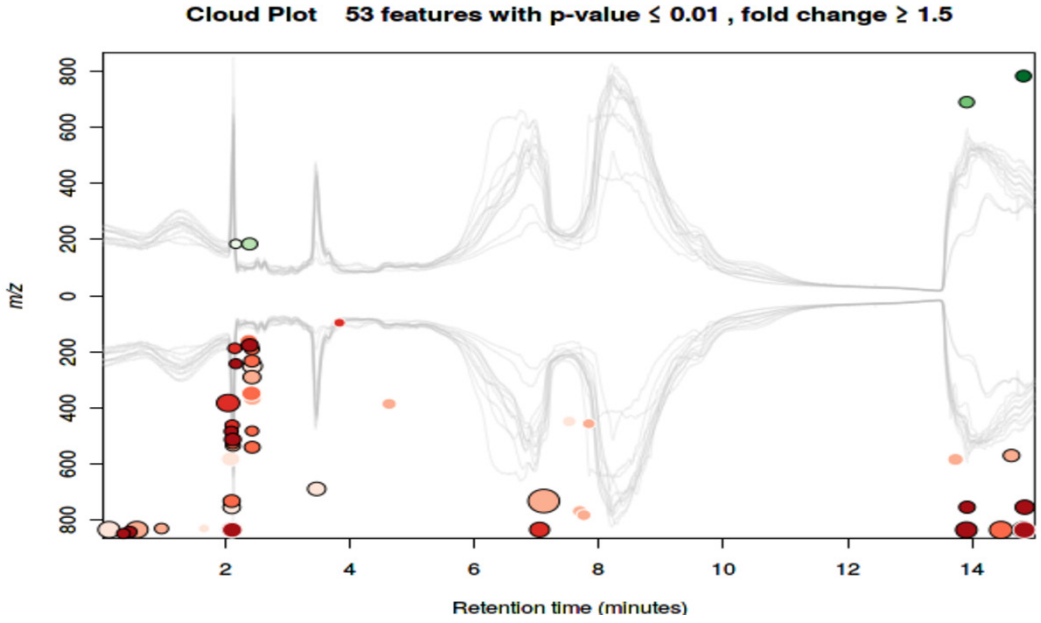

FIGURE 5 - Cloud plot of 53 different metabolites in the drug group compared to the control group (p-value ≤0.01, fold change ≥1.5).

TABLE I - List of the significantly different metabolites identified in the drug group compared to the control group

Metabolites	Fold	P value	Regulation	m/z med	Rt med
Indoleacrylic Acid	1.7	0.00240	DOWN	188.0716	2.16
Arachidonic Acid	1.8	0.00332	DOWN	304.4669	2.12
LysoPC(22:5(4Z,7Z,10Z,13Z,16Z)/0:0)	2.2	0.00741	DOWN	570.3582	14.62
Palmitic Acid	18.4	0.00793	DOWN	732.5572	7.12
Thromboxane A2	1.5	0.00885	DOWN	352.4651	4.68
Choline	1.5	0.00971	DOWN	104.1079	3.38
Normetanephrine	1.5	0.00978	UP	184.0954	2.18
Homocarnosine	1.8	0.00227	DOWN	280.0916	3.57
PC(18:1(9Z)/18:1(9Z))	10.4	0.00172	DOWN	786.1134	14.80
LysoPE(0:0/20:1(11Z))	2.5	0.00152	DOWN	508.3431	14.93
11H-14,15-EETA	4.1	0.00199	DOWN	336.4657	11.23
Uridine 5'-diphosphate	1.8	0.00207	DOWN	442.9630	7.85
Thromboxane B2	2.7	0.00583	DOWN	368.4645	8.48
23S,25-dihydroxyvitamin D3	1.6	0.00624	DOWN	416.6365	5.03
L-Tryptophan	1.4	0.00931	UP	204.2252	2.99
Prostaglandin D2-1-Glyceryl Ester	2.9	0.00272	UP	426.5436	2.13
L-Kynurenine	3.3	0.00253	UP	204.2252	13.4
L-Methionine	1.8	0.00284	DOWN	150.0592	2.15
1,25-Dihydroxyvitamin D3-26,23-lactone	3.1	0.00464	DOWN	444.6249	5.12
Calcitriol	4.7	0.00468	DOWN	401.2881	3.84
3-methoxy-4-hydroxyphenylpyruvate	1.8	0.00497	DOWN	194.0335	7.87
Calcidiol	1.9	0.00476	DOWN	400.6371	4.71
L-Phenylalanine	1.6	0.00527	DOWN	165.1891	2.12
Nornicotine	1.7	0.00782	DOWN	188.0716	2.16
Histamine Phosphate	2.1	0.00454	DOWN	308.0403	3.68
Uridine	1.7	0.00678	DOWN	267.0584	3.66
Tyrosine Methylester	1.5	0.00580	DOWN	195.2151	2.12
5-Aminoimidazole Ribonucleotide	1.8	0.00981	DOWN	296.0665	2.17
Beta-Guanidinopropionic acid	1.5	0.00284	DOWN	132.0776	2.17
Calcitroic Acid	1.7	0.00499	DOWN	374.5137	14.91
Nervonyl Carnitine	1.7	0.00467	UP	102.1286	7.89
β-nicotinamide D-ribonucleotide	1.6	0.00334	DOWN	336.0729	2.13
2-(a-Hydroxyethyl)thiamine diphosphate	1.9	0.00472	DOWN	469.0712	2.12

TABLE I - List of the significantly different metabolites identified in the drug group compared to the control group

Metabolites	Fold	P value	Regulation	m/z med	Rt med
Thyroxine glucuronide	4.2	0.00743	UP	991.6758	7.91
N1-acetyl-N2-formyl-5-methoxykynuramine	1.8	0.00384	DOWN	248.0928	2.12
Retinyl Ester	2.0	0.00425	DOWN	313.4538	5.14
3-Iodothyronamine	2.3	0.00457	UP	394.9757	2.03
L-Cysteine	2.6	0.00478	DOWN	240.3921	3.22
L-Leucine	1.8	0.00635	DOWN	132.1026	3.59
L-Isoleucine	1.9	0.00756	DOWN	131.1742	3.21
Beta-Alanine	3.7	0.00426	DOWN	89.0932	3.11
N-Acetyl-L-Alanine	1.8	0.00685	DOWN	131.1299	3.59
Oxaloacetic Acid	2.5	0.00512	DOWN	132.0716	4.49
L-Valine	3.1	0.00779	DOWN	117.1542	3.28
Sphinganine	1.5	0.00894	DOWN	341.2684	4.68
L-Histidine	2.1	0.00921	UP	155.1546	14.51
Triiodothyronine Sulfate	2.9	0.00842	DOWN	366.8839	2.44
Oxidized glutathione	1.7	0.00926	UP	612.6317	0.86
7alpha-Hydroxy-3-oxo-4-cholestenoate	3.1	0.00348	DOWN	430.6291	3.52
Decanoylcarnitine	9.4	0.00428	DOWN	315.4482	14.83
PE(18:2(9Z,12Z)/22:2(13Z,16Z))	10.2	0.00615	DOWN	796.5826	14.83

Metabolic pathways which could be affected by the metabolic alterations were determined by the pathway analysis. 53 metabolites reported in Table I were imported to MetaboAnalyst 4.0 Software, and detailed metabolic pathway analysis of these metabolites was performed. MetaboAnalyst 4.0 Software was used for metabolic pathway analyzes of the identified metabolites. The relevant pathways and their effect values are shown in Figure 6. According to the data obtained from MetaboAnalyst 4.0 Software, it was observed that 38 different metabolic pathways may be affected due to the significant difference observed in 53 metabolites. Metabolites that were significantly altered, involved in the pathways of which have -log p values higher than 2 were discussed below (Significantly identified metabolites/total metabolites in an individual pathway): 8/48 aminoacyl tRNA biosynthesis, 3/8 valine, leucine, and ısoleucine biosynthesis, 2/20 TCA (citrate) cycle, 4/36 arachidonic acid metabolism, 4/36 glycerophospholipid metabolism, 3/19 pantothenate and CoA biosynthesis, 1/4 phenylalanine, tyrosine, and tryptophan biosynthesis, 1/16 retinol metabolism, 1/16 histidine metabolism, 2/22 pyruvate metabolism, 1/5 linoleic acid metabolism and 2/28 cysteine and methionine metabolism.

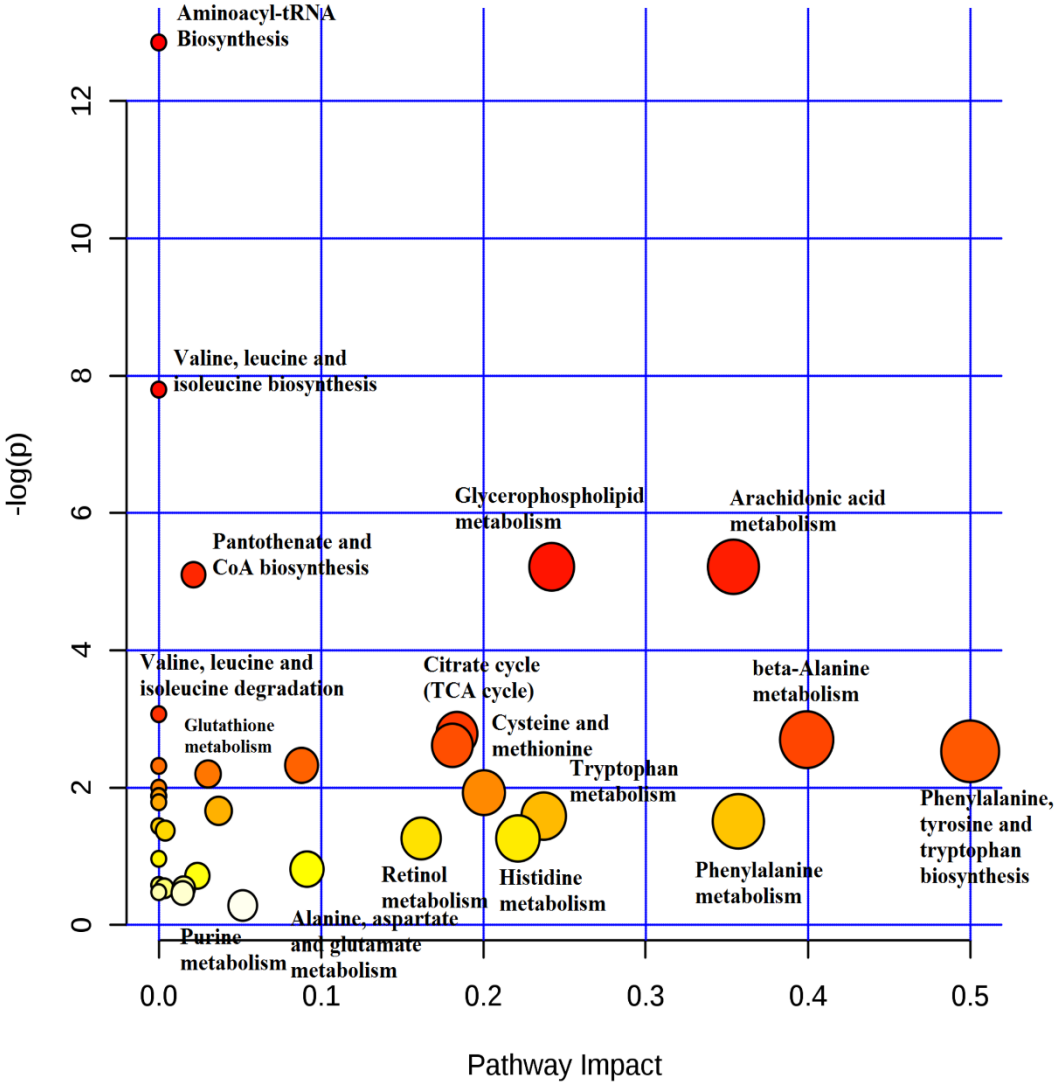

FIGURE 6 - Impact analysis for pathways created by MetaboAnalyst online: main pathways altered in the drug group vs the control group.

DISCUSSION

Antimuscarinic agents have been the basis of OAB treatment in recent years. While antimuscarinics act by detecting M_3-receptors on detrusor smooth muscle cells, they can also act on sensory and central pathway receptors (Abrams *et al.*, 2006; Finney *et al.*, 2006). Muscarinic receptors are widely found in the body, including the central nervous system, eyes, salivary glands, and gastrointestinal tract. Therefore, antimuscarinic exposure may lead to changes in many biological events on metabolism. If OAB is ignored and treated, it can progress and become chronic. To prevent this, it may be necessary to use solifenacin succinate for a long time (Kim, Lee, 2016). In clinical studies conducted in the past, it has been observed that the use of solifenacin succinate for an average of 6.5 months is effective in eliminating symptoms. Due to prolonged exposure to solifenacin, common side effects are observed as a result of parasympathetic stimulation. Side effects play an important role (23.7%) in patients' termination of anticholinergic therapy. Side effects such as dry eyes, dry mouth, and blurred vision, headache, constipation, urinary retention, restlessness, confusion, and hallucinations

have been reported to cause discontinuation of treatment. It has also been stated that solifenacin may accelerate acute narrow-angle glaucoma and acute urinary retention (Krhut *et al.*, 2014; Ali *et al.*, 2019).

-Omics technologies enable us to have detailed information about complex biological systems by explaining the relationships, functions, and differences of the molecules in tissue and biological fluids with a general approach. In this study, the metabolomics approach was applied to rats to obtain a small representation of the metabolic profiles of solifenacin succinate in humans. Our aim is to observe metabolome profiles of the chronic exposure of daily recommended solifenacin dose on rat metabolism. For this purpose, the daily rat dose was calculated by simulating the recommended daily solifenacin dose (Astellas, 2009) for the average human weight (62 kg) (Walpole *et al.*, 2012) to the average rat weight. The rats in the two groups were weighed one by one and the average rat weight was found to be 332.4 g. The contents of 10 Kinzy Capsules (5 mg solifenacin succinate) were homogeneously mixed. This mixture was diluted with water and drug solutions containing 0.026 mg solifenacin succinate in 1 mL of water were administered orally for each rat. (Doroshyenko, Fuhr 2009; Yamaguchi *et al.*, 2007) In this first pharmacometabolomics study, significant metabolic changes occurred in the experimental group with prolonged exposure to solifenacin succinate.

In LC-Q-TOF/MS/MS analysis of plasma samples, 4440 different features were detected. Among these features, 53 metabolites, whose fold change is > 1.5 and p-value is $p \leq 0.01$, are identified by HMDB and METLIN (Table I). The identified 53 metabolites are involved in 38 metabolic pathways in rat metabolism.

Changes in the aminoacyl-tRNA biosynthesis determined in the metabolic pathway analysis led to inhibition of the transport and synthesis of l-leucine, l-isoleucine, l-valine, and 2- (a-hydroxyethyl) thiamine diphosphate amino acids. As a result, the plasma levels of the relevant amino acids decreased, causing changes in valine, leucine, and isoleucine biosynthesis. These amino acids are involved in the synthesis of muscle proteins, the proliferation of muscle cells, and muscle contraction. The increased amount of l-leucine, l-isoleucine, and l-valine is directly related to the proliferation of prostate tissue and bladder contraction (Stark *et al.*, 2012; Hutter *et al.*, 2013; Hongpaisan, 2000). With the use of solifenacin in benign prostatic hyperplasia and OAB, prostate enlargement and frequent contraction of the bladder muscle are eliminated (Lee *et al.*, 2011). Our study proves that these therapeutic effects of solifenacin are caused by the reduction of l-leucine, l-ısoleucine, l-valine amino acids. Also, l-leucine activates the release of l-alanine from the muscles. This reduction of l-leucine was confirmed by the decrease in plasma l-alanine level in our study (Mero, 1999).

Due to the decreases in l-phenylalanine and l-tryptophan plasma amounts, the drug may be thought to cause inhibition of protein synthesis. Inhibition of protein synthesis, one of the most important biological events for metabolism, can lead to psychological symptoms, inhibition of hormone synthesis, and disruption of the epithelial structure by preventing the growth and development of cells (Azmitia, 2001). Low l-phenylalanine plasma level slows growth. Because if there is not enough l-phenylalanine in metabolism, protein synthesis is impaired and growth may be adversely affected.

It has been reported in the literature that the interaction between glutamate and acetylcholine has many physiological consequences. One of them is that glutamate has inhibitory effects on peripheral organs such as salivary glands (Shida *et al.*, 1995). Plasma levels of oxidized glutathione, l-glutamate, l-histidine, beta-alanine, n-acetyl-l-alanine, and l-aspartate significantly changed with solifenacin exposure. Our study has shown that changes in the amounts of these amino acids disrupt the functioning of histidine and beta-alanine metabolism. Due to the alteration of histidine and beta-alanine metabolism, the metabolism of alanine, aspartate, and glutamate has also changed. Thus, plasma l-glutamate level increased in rats. It has been enlightened by our study that the side effects of solifenacin are caused by the increased glutamate level.

Also, thiamine metabolism was affected by the decrease in plasma 2-(alpha-hydroxyethyl) thiamine diphosphate level and the increase in plasma l-glutamine level. Altered thiamine metabolism influenced pyridoxal 5'-phosphate synthesis. Decreased

pyridoxal 5'-phosphate plasma level decreased B6 vitamin synthesis due to pyridoxal synthesis (Girard, Matte, 1999). It has been reported that vitamin B6 deficiency affects the ocular surface and causes dry eyes (Aragona *et al*., 2013). Our study has shown that dry eye and visual blurring side effects occur with alteration of thiamine metabolism.

In the literature, it has been reported that l-phenylalanine inhibits circular smooth muscles by activating the extracellular calcium-sensing receptor (CaSR) in the intestines (Gwynne *et al*., 2017). It can be thought that the decrease in l-phenylalanine level causes constipation from the side effects of solifenacin. Because it is known that l-phenylalanine inhibits the contraction of intestinal circular smooth muscle. Our study has confirmed this information. In addition, the decrease in l-methionine and l-cysteine plasma levels indicates a decrease in vitamin B12 synthesis. It has been reported that vitamin B12 deficiency also leads to constipation and weight loss (Sturtzel *et al*., 2010).

The decrease in plasma levels of calcidiol, 1,25-dihydroxyvitamin D3-26,23-lactone and 23S,25-dihydroxyvitamin D3 metabolites, which are intermediate forms in vitamin D metabolism, shows that solifenacin succinate negatively affects vitamin D synthesis. It has been reported in the literature that vitamin D deficiency affects all systems of the body and causes many diseases (Holick, 2007). Dry eye and blurred vision symptoms occur with the disruption of the nervous system from these systems (Yildirim *et al*., 2016; Krhut *et al*., 2014; Ali *et al*., 2019). Our study confirmed the association of dry eye and blurred vision, two of the most common side effects of solifenacin, with vitamin D as well as thiamine. It can be thought that these two side effects can be eliminated with vitamin D supplements.

Active calcium absorption from the intestine is dependent on an adequate plasma level of 1,25-dihydroxyvitamin D3. Calcium activates the contraction in smooth muscles. Vitamin D helps the absorption and use of calcium into the body. Low vitamin D level reduces smooth muscle contraction that starts with calcium (Lips, 2006). The relationship between solifenacin relaxation of smooth muscles and vitamin D plasma level has been elucidated by our study.

Decreased levels of arachidonic acid, phosphatidylcholine, 14,15-epoxyeicosatrienoic acid (14,15-EETA), and thromboxane A2 metabolites in rats treated with solifenacin succinate show that the synthesis of arachidonic acid is impaired. Arachidonic acid, a long-chain unsaturated fatty acid, provides fluidity and flexibility to the cell as an integral component of the cell membrane. Therefore, arachidonic acid is essential for the functioning of all cells and especially nervous, skeletal, and immune systems. Drying, itching, rash and sensitivity may occur on the skin with solifenacin exposure. In addition, drug exposure can also cause loss of appetite (Krhut *et al*., 2014; Ali *et al*., 2019). These side effects may be related to decreased plasma levels of arachidonic acid, phosphatidylcholine, 14,15-epoxyeicosatrienoic acid (14,15-EETA), and thromboxane A2 metabolites as a result of drug exposure. Because it is known that linoleic acid and alpha-linolenic acid synthesis decreases due to arachidonic acid deficiency. It has been reported that the aforementioned skin and appetite-related symptoms have been observed with a deficiency of linoleic acid and alpha-linolenic fatty acids (Le *et al*., 2009).

CONCLUSIONS

In this study, the metabolomics approach was applied to rats to obtain a small representation of the metabolic profiles of solifenacin succinate in humans. Our study revealed metabolic differences between two groups such as drug and control at the semi-quantitative level. Our study showed that plasma levels of 53 metabolites and 38 metabolic pathways were altered by exposure to solifenacin succinate. Changes in these metabolic pathways should be considered in the treatment of OAB with solifenacin succinate. If patients using solifenacin succinate experience visual impairment, vitamins D, B6, and B12 may be recommended as supplements to avoid side effects. If constipation develops with exposure to solifenacin succinate, either a laxative may be recommended or a phenylalanine supplement may be given.

The therapeutic and side effects of solifenacin succinate reported in the literature were explained at the metabolite level. Interpretation of the identified

metabolites and metabolic pathways has provided a better understanding of the consequences of these effects. The information presented in the literature and those presented in this study will help scientists to better understand OAB treatment with low adverse effects and high therapeutic efficacity.

This first pharmacometabolomics study reported in the literature for solifenacin succinate will lead to perform new toxicological and metabolomics studies for other drugs and to investigate the effects of drugs on metabolic mechanisms and pathways.

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AUTHOR CONTRIBUTIONS

TCA and YK designed the study. BE with the aid of OS and TCA has conducted the animal experiments. TCA and OS analyzed the data. All authors co-wrote the paper and approved the submission of the final version of the manuscript.

ETHICS APPROVAL

Animal experiments were approved by Atatürk University Local Animal Care Committee. (HADYEK 75296309-050.01.04-E.1700196080)

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest to disclose.

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