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Altered Levels of Gene Expression of Drug Metabolism Enzymes in Rat Brain Following Kainic Acid Treatment

Ayfer Yalcin[1], Ezgi Turunc[2], Guliz Armagan[1], Lutfiye Kanit[3]
[1]Department of Biochemistry, Faculty of Pharmacy, Ege University, Izmir, Turkey
[2]Department of Biochemistry, Faculty of Pharmacy, Izmir Katip Celebi University, Izmir, Turkey
[3]Department Of Physiology, Faculty Of Medicine, Ege University, Izmir, Turkey

Corresponding Author Information
Ayfer Yalcin
https://orcid.org/0000-0003-0407-3218
ayfer.yalcin@ege.edu.tr
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ABSTRACT
Objectives: Previous studies have shown that gene expressions can be regulated in the hippocampus of rats after seizures induced by kainic acid (KA). The purpose of this study was to examine the potential regulatory impact of KA administration on gene expression levels of enzymes responsible for drug metabolism in the rat hippocampal tissue.
Materials and Methods: Rats received intraperitoneal injections of KA and saline at a dose of 10 mg/kg each. Behavioral changes were observed in experimental animals following the administration of KA. Four hours after receiving treatments, all rats were decapitated, and the brains were removed. Hippocampal tissues were used for total RNA isolation, and cDNA synthesis was achieved by reverse transcription PCR. Gene expression levels of enzymes responsible for drug metabolism were determined by qPCR using the RT^2 Profiler PCR Array Rat Drug Metabolism PCR array system containing the relevant primers for a total of 84 genes. The gene expression levels of drug-metabolising enzymes (DMEs) were quantified using comparative Ct (2-ΔΔCt) method. Student's t-test was used for data analysis.
Results: Our results indicate that KA treatment caused significant changes in the gene expression levels of metallothionein 3 (Mt3), glucose phosphate isomerase (Gpi), ATP-binding cassette protein C1 (Abcc1), cytochrome P450 enzymes (Cyp2c6v1, Cyp3a23/3a1, Cyp2c7), glutathione peroxidase 1, 4 and 5 (Gpx1, Gpx4, Gpx5), glutamic acid decarboxylase 1 and 2 (Gad1, Gad2), paraoxonase 2 (Pon2), carbohydrate sulfotransferase 1 (Chst1), glutathione S-transferases (Gsta3, Gstm1, Gstm4), microsomal glutathione S-transferase 3 (Mgst3), carboxylesterase 2C (Ces2c), fatty acid amide hydrolase (Faah), pyruvate kinase-muscle (Pkm2), arachidonate 5-lipoxygenase (Alox5), apolipoprotein E (Apoe), cytochrome b5 reductase 5 (Cyb5r5), xanthine dehydrogenase (Xdh), N-acetyltransferase 1 (Nat1), glucokinase regulator (Gckr), hexokinase 2 (Hk2), myristoylated alanine rich protein kinase C substrate (Marcks), and stannin (Snn) in hippocampus compared to control ($p<0.05$).
Conclusion: As a conclusion, it can be said that the seizure activity triggered by KA has the potential to change the gene expression levels of the enzymes responsible for drug metabolism in the hippocampus of rats.
Key words: Kainic acid, status epilepticus, hippocampus, drug metabolism, gene expression, PCR array

INTRODUCTION

Kainic acid is an analog of glutamate, an excitotory amino acid. The treatment of KA to rodents results in seizures and neoronal death in specific brain regions such a hippocampus.[1-3] It is known that neuropathological changes induced in the brain by KA are similar to the changes deteceted in the hippocampus region of patients with temporal lobe epilepsy (TLE).[4]
Different enzyme classes that are in charge of the biochemical alteration of medicinal compounds participate in drug metabolism. Drug-metabolizing enzymes (DMEs) are also found in extrahepatic tissues, such as the brain, despite the liver being the primary organ of metabolism in the body.[5,6]
Prior research has demonstrated that KA-induced seizures or status epilepticus (SE) can alter gene expression in the rat brain.[7-10] There appear to be few studies examining the impact of KA administration on DMEs. Conducting research on potential genetic controls of KA-induced SE on DMEs in rat brain was deemed advantageous in this context. This approach might also be helpful for identifying the gene profile of potential molecular targets in KA-induced seizures. According to these stated goals, the objective of our investigation was

to determine how the KA administration affected the relative expression levels of DMEs, which include drug transporters, P-glycoproteins, and Phase I and Phase II drug metabolizing enzymes.

MATERIALS AND METHODS

Animals and in vivo treatments

Twelve male adult Sprague-Dawley rats weighed 200-230g were used for the present study. The rats were housed under the identical laboratory settings of lighting (14:10 h lightdark) and temperature (24±2°C) and had free access to normal laboratory food and tap water. Every attempt was made to minimise animal suffering and the number of animals employed. The procedure for the experiments was authorized by XXX University, Faculty of Pharmacy, Experimental Animal Ethics Committee (2006/6-1).

Kainic acid [2-carboxy-4-isopropenyl-pyrrolidin-3-acetic acid] was obtained from Ocean Products International (Canada). Rats received intraperitoneal injections of KA and saline at a dose of 10 mg/kg each according to previous studies.[10,11] Behavioral changes were observed in experimental animals following the administration of KA. KA promotes seizures and selective excitotoxic cell death, primarily in the limbic structure, in rodents when administered systemically.[2] Once the KA was administered, it resulted in a series of behavioral alterations that were clearly characterized. After 45 minutes, the rats' rigidity and immobility were replaced by "staring spells," which were then followed by wet dog shakes and repetitive head nodding, and finally by rearing and falling. A generalized tonic-clonic seizure with ongoing convulsions eventually developed in the rats. Four hours after receiving treatments, all rats were decapitated, The brains were removed and the hippocampus tissues were dissected. The effect of KA on the expression of drug metabolizing enzymes was investigated using the RT^2 Profiler PCR Array Rat Drug Metabolism (SABiosciences, Qiagen, Maryland, USA) in accordance with the manufacturer's guidelines. Each array contained 5 housekeeping genes and a panel of 84 target genes related to drug metabolism and transport.

The hippocampal tissues were used for total RNA isolation with Trizol (Invitrogen, USA) followed by phenol chloroform extraction and isopropanol precipitation chloroform.[12] The complementary DNA (cDNA) was synthesized from total RNA by reverse transcription PCR RT^2 PCR array first strand kit. The real-time PCR mixture, which contained RT^2 master mix, nuclease-free H_2O, and cDNA, was loaded onto a PCR plate. PCR amplification was conducted with initial step at 95 °C for 10 min and followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Threshold cycles were detected for all genes and the data obtained was examined using PCR Array Data Analysis Software (SABiosciences, USA). The gene levels of DMEs were determined using comparative Ct ($2^{-\Delta\Delta Ct}$) method.[13,14] Gene expressions were normalized using β-actin (Actb), ribosomal protein large P1 (Rplp1), ribosomal protein L13A (Rpl13a), hypoxanthine phosphoribosyltransferase-1 (Hprt1), and lactate dehydrogenase A (Ldha) as reference genes which included in the PCR array kit. The data was evaluated using the Student's t-test, and $p<0.05$ was considered statistically significant.

RESULTS

Mt3, Abcc1, and Gpi gene expression levels were considerably lower after KA treatment compared to control ($p<0.05$) (Table 1, Fig. 1). When compared to controls, KA administration significantly upregulated Cyp2c6v1, Cyp3a23/3a1, and downregulated Cyp2c7 gene expressions ($p<0.05$) (Table 2, Fig. 1). When compared to the control, KA administration significantly changed the expression of the genes Gpx1, Gpx5, Pon2, Chst1, Gsta3, Mgst3, and lowered the expression of the genes Ces2c, Gad1, Gad2, Gpx4, Faah, Pkm2, Alox5, Apoe, Cyb5r5, Gstm1, Gstm4, Xdh, Nat1, Gckr, Hk2, Marcks, and Snn ($p<0.05$) (Table 3, Fig. 2).

DISCUSSION

Effects of KA on Drug Transporters and P-glycoproteins

The metallothionein isoforms (Mt1, Mt2, and Mt3) may be controlled differentially in the rat brain depending on their roles in cell type-dependent cellular responses to KA-induced damage.[15] In line with this finding, we found that KA treatment reduced the level of Mt3 expression in the hippocampus. The enzyme Gpi, also known as neuroleukin (NLK), is reported to be involved in metabolic activities. It has also been shown that focal ischemia-induced brain injury results in a decrease in Gpi protein expression levels.[16] In light of this, it can be concluded that the lower levels of Gpi found in our study may be linked to a potential metabolic constraint that could emerge from KA-induced neuronal injury.

Effects of KA on Phase I Drug-Metabolizing Enzymes

The cytochrome P450 superenzyme family, particularly in the liver, is reported to be involved in biotransformation activities in the brain.[17] Disruptions in CYP-related biotransformation systems in the brain have been hypothesized to be a factor in metabolic decline or the development of drug toxicity.[18] Additionally, it has been observed that a number of antiepileptic medications are known to induce CYP isoenzymes.[19]

Our findings indicate that after KA treatment, a major portion of P450 enzymes showed statistically insignificant high or low results in terms of gene expression levels. But within the parameters of our findings, it was also

observed that KA treatment markedly upregulated Cyp2c6v1, Cyp3a23/3a1, and Cyp2c7 expression levels. In light of this, it is possible to state that KA administration may regulate the gene expression of P450 enzymes in the hippocampus of rats.

Effects of KA on Phase II Drug-Metabolizing Enzymes

The enzymes glutathione-S-transferase (Gsts), which use glutathione in their activities, are known to be involved in detoxification processes and to provide protection against oxidative stress. In a prior study, it was found that the expression of Gsta4 increased in correlation with neuronal damage following oxidative stress caused by substances like paraquat or zinc.[20] In this case, the increase in Gsta4 gene expression levels detected in our study can be explained by the fact that KA is also an agent that causes the emergence of reactive oxygen species (ROS).[11]

It has been reported that glutathione peroxidase enzymes provide a protection against various neurotoxic agents.[21] In addition, it has been reported that there was an increase in Gpx1 levels in the brain tissue of mesial temporal lobe epilepsy patients.[22] In this context, in our study, gene expression levels of all GPx enzymes, except Gpx4, were increased following KA treatment, which is a neurotoxic agent. However, the increase in gene expression levels of only Gpx1 and Gpx5 among these enzymes was relatively lower.

In a microarray study aiming to reveal the transcriptome profile of hippocampal CA1 region after preconditioning due to early life seizure, it was reported that the glutamic acid decarboxylase 1 (GAD1) gene was overexpressed within the scope of neuroprotective genes after 3x KA treatment.[23] In our study, GAD1 and GAD2 gene expression levels were found to be significantly reduced in the hippocampus tissue after KA administered at a dose of 10 mg/kg to rats, which may be an indicator of the regulatory effect of KA on these genes.

In a study on mice, it was reported that overexpression of Cyb5r3 reduced oxidative damage, improved mitochondrial function and inhibited proinflammatory pathways.[24] According to our results, Cybr5 gene expression was found to be significantly decrease due to the potential neurotoxic effects of KA in the hippocampus.

It has been suggested that PON2 is a neuroprotective enzyme due to its antioxidant and anti-inflammatory properties.[25] According to our findings PON1, PON2, and PON3 gene expression levels all upregulated by KA treatment, however only PON2's increase reached a statistically significant level.

It has been stated that Apolipoprotein E (Apoe) plays a role in various CNS disorders by modulating microglial activation.[26] There have also been some studies showing that Apoe can modulate hippocampal damage induced by KA.[27] In addition, it has been suggested that Apoe deficiency increases microglial activation and hippocampal damage in mice exposed to KA.[26] Consistent with this observation, our results suggest that decreased Apoe levels may be an indicator of neuronal damage that may be caused by KA in the hippocampus.

In our study, all pyruvate kinase enzyme levels were found to be low, which is critical for fundamental metabolic pathways. However, only Pkm2 levels were found to be significantly reduced among these enzymes, which could be explained by the neurotoxic effect of KA on the metabolic pathways.

In a transgenic mouse study, it was found that KA administration increased the level of chondroitin 6-sulfation in the hippocampus and cerebral cortex, and that transgenic mice overexpressing chondroitin 6-sulfate chains were more sensitive to KA-induced seizures than wild-type mice.[28] The Chst1 enzyme is also known to be involved in the sulfation of carbohydrates. In this context, increased Chst1 levels, as detected in our study, could indicate neuronal damage caused by KA.

A previous study found that a 25 mg/kg dose of KA increased Marcks protein expression in microglial cells.[29] In our study, it was observed that KA administration at a dose of 10mg/kg decreased Marcks gene expression levels in the hippocampus tissue, which can be interpreted as a dose-dependent effect.

It has been proposed that Snn is a protein involved in mitochondrial responses as part of the mechanisms that cause brain damage.[30] In our study, we found a significant decrease in Snn gene expression levels after KA-induced seizures, which is thought to be an indication of possible mitochondrial damage caused by KA.

It was previously reported in a study with Faah enzyme inhibitors that some specific inhibitors protect against brain damage after KA treatment.[31] However, in our study, there was a decrease in Faah gene expression levels after KA treatment, which is thought to be related to the level of neuronal damage caused by KA.

Study Limitations

Our research has some limitations. By adding additional animals to the experimental groups, we could improve the efficacy of our gene expression analysis results. We were unable to examine the amounts of proteins or the activity of the enzymes involved in drug metabolism. Finding gene expression levels may not always provide an accurate estimate of a protein's concentration. Thus, for a more thorough examination, protein quantification and enzyme activity measurements are needed.

CONCLUSION

In conclusion, our findings suggest that KA treatment may alter gene expression levels of enzymes involved in drug metabolism in rat hippocampus tissue. Furthermore, our findings may contribute to the gene expression

profile previously revealed after KA treatment in the context of various neurotoxic or neurodegenerative conditions.

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Declaration of Conflicting Interests
The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Table 1. Regulated levels of drug transporters and P-glycoproteins gene expressions in the hippocampus of rats after KA-treatment. Gene expression levels are stated as n-fold change normalized to control group. Each sample is tested in triplicate. **p<0.05.*

Gene Symbol	Drug Metabolism Enzymes	Expression Levels
Drug Transporters and P-glycoprotein family		
Mt3	Metallothionein 3	0,332 ± 0,014*
Abcb1b	ATP-binding cassette, subfamily B (MDR/TAP), member 1B	1,014 ± 0,075
Abcb1a	ATP-binding cassette, sub-family B (MDR/TAP), member 1A	0,889 ± 0,116
Abcb4	ATP-binding cassette, subfamily B (MDR/TAP), member 4	1,357 ± 0,252
Abcc1	ATP-binding cassette, subfamily C (CFTR/MRP), member 1	0,655 ± 0,014*
Abp1	Amiloride binding protein 1 (amine oxidase, copper-containing)	2,219 ± 0,791
Gpi	Glucose phosphate isomerase	0,297 ± 0,017*

Table 2. Regulated levels of Phase I metabolizing enzymes (P450 Family) in the hippocampus of rats after KA-treatment. Gene expression levels are stated as n-fold change normalized to control group. Each sample is tested in triplicate. **p<0.05.*

Gene Symbol	Drug Metabolism Enzymes	Expression Levels
Phase I Metabolizing Enzymes-P450 Family		
Cyp17a1	Cytochrome P450, family 17, subfamily a, polypeptide 1	3,227 ± 1,893
Cyp19a1	Cytochrome P450, family 19, subfamily a, polypeptide 1	0,012 ± 0,005
Cyp1a1	Cytochrome P450, family 1, subfamily a, polypeptide 1	3,580 ± 1,772
Cyp1a2	Cytochrome P450, family 1, subfamily a, polypeptide 2	2,603 ± 1,468

Cyp1b1	Cytochrome P450, family 1, subfamily b, polypeptide 1	1,000 ± 0,095
Cyp27b1	Cytochrome P450, family 27, subfamily b, polypeptide 1	2,567 ± 1,324
Cyp2b15	Cytochrome P450, family 2, subfamily b, polypeptide 15	0,928 ± 0,086
Cyp2b6	Cytochrome P450IIB3	0,986 ± 0,099
Cyp2c13	Cytochrome P450, family 2, subfamily c, polypeptide 13	1,717 ± 0,614
Cyp2c6v1	Cytochrome P450, family 2, subfamily c, polypeptide 6	2,969 ± 0,235*
Cyp2c7	Cytochrome P450, family 2, subfamily c, polypeptide 7	0,775 ± 0,049*
Cyp2e1	Cytochrome P450, family 2, subfamily e, polypeptide 1	1,591 ± 0,487
Cyp3a23/3a1	Cytochrome P450, family 3, subfamily a, polypeptide 23/polypeptide 1	3,706 ± 0,555*
Cyp4b1	Cytochrome P450, family 4, subfamily b, polypeptide 1	0,012 ± 0,006

Table 3. Regulated levels of Phase II metabolizing enzymes in the hippocampus of rats after KA-treatment. Gene expression levels are stated as n-fold change normalized to control group. Each sample is tested in triplicate. *$p<0.05$.

Gene Symbol	Drug Metabolism Enzymes	Expression Levels
Phase II Metabolizing Enzymes		
Lipoxygenases		
Alox15	Arachidonate 15-lipoxygenase	1,866 ± 0,539
Alox5	Arachidonate 5-lipoxygenase	0,012 ± 0,004*
Apoe	Apolipoprotein E	0,083 ± 0,009*
Oxidoreductases		
Blvra	Biliverdin reductase A	2,657 ± 1,215
Blvrb	Biliverdin reductase B (flavin reductase (NADPH)	2,732 ± 1,483
Cyb5r5	Cytochrome b5 reductase 5	0,255 ± 0,021*
Gsr	Glutathione reductase	2,639 ± 1,367
Mthfr	Methylenetetrahydrofolate reductase (NAD(P)H)	2,313 ± 1,088
Nos2	Nitric oxide synthase 2, inducible	2,317 ± 1,002
Nos3	Nitric oxide synthase 3, endothelial cell	2,462 ± 1,245
Nqo1	NAD(P)H dehydrogenase, quinone 1	3,031 ± 1,626
Srd5a1	Steroid-5-alpha-reductase, alpha polypeptide 1 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 1)	2,014 ± 0,997
Xdh	Xanthine dehydrogenase	0,012 ± 0,005*
Paraoxonases		
Pon1	Paraoxonase 1	4,922 ± 2,096
Pon2	Paraoxonase 2	1,505 ± 0,039*
Pon3	Paraoxonase 3	1,717 ± 0,567
Glutathione S-transferases		
Chst1	Carbohydrate (keratan sulfate Gal-6) sulfotransferase 1	1,329 ± 0,012*
Gsta3	Glutathione S-transferase A3	1,301 ± 0,009*
Gsta4	Glutathione S-transferase alpha 4	2,514 ± 1,494
Gstm1	Glutathione S-transferase mu 1	0,753 ± 0,021*
Gstm2	Glutathione S-transferase mu 2	2,144 ± 1,128
Gstm3	Glutathione S-transferase mu 3	0,742 ± 0,283
Gstm4	Glutathione S-transferase mu 4	0,763 ± 0,017*
Gstm5	Glutathione S-transferase, mu 5	1,141 ± 0,135
Gstp1	Glutathione S-transferase pi 1	1,125 ± 0,112
Gstt1	Glutathione S-transferase theta 1	4,258 ± 2,999
Mgst1	Microsomal glutathione S-transferase 1	2,657 ± 1,386
Mgst2	Microsomal glutathione S-transferase 2	4,469 ± 3,015
Mgst3	Microsomal glutathione S-transferase 3	1,347 ± 0,016*
Transferases		
Nat1	N-acetyltransferase 1	0,012 ± 0,008*
Comt1	Catechol-O-methyltransferase	1,840 ± 0,784

Ggt1	Gamma-glutamyltransferase 1	2,603 ± 1,372
Other Genes Related to Drug Metabolism		
Arnt	Aryl hydrocarbon receptor nuclear translocator	1,613 ± 0,493
Ahr	Aryl hydrocarbon receptor	1,548 ± 0,401
Asna1	ArsA arsenite transporter, ATP-binding, homolog 1 (bacterial)	0,914 ± 0,065
Gckr	Glucokinase (hexokinase 4) regulator	0,004 ± 0,002*
Marcks	Myristoylated alanine rich protein kinase C substrate	0,753 ± 0,027*
Smarcal1	Swi/SNF related matrix associated, actin dependent regulator of chromatin, subfamily a-like 1	1,198 ± 0,126
Snn	Stannin	0,637 ± 0,025*
Carboxylesterases		
Ces1e	Carboxylesterase 1E	2,695 ± 1,571
Ces2c	Carboxylesterase 2C	0,986 ± 0,007*
Decarboxylases		
Gad1	Glutamic acid decarboxylase 1	0,946 ± 0,005*
Gad2	Glutamic acid decarboxylase 2	0,387 ± 0,034*
Dehydrogenases		
Adh1	Alcohol dehydrogenase 1 (class I)	3,011 ± 1,568
Adh4	Alcohol dehydrogenase 4 (class II), pi polypeptide	3,272 ± 1,923
Alad	Aminolevulinate, delta-, dehydratase	0,500 ± 0,396
Aldh1a1	Aldehyde dehydrogenase 1 family, member A1	1,765 ± 0,582
Hsd17b1	Hydroxysteroid (17-beta) dehydrogenase 1	2,099 ±1,115
Hsd17b2	Hydroxysteroid (17-beta) dehydrogenase 2	3,011 ± 1,679
Hsd17b3	Hydroxysteroid (17-beta) dehydrogenase 3	2,297 ± 1,107
Glutathione Peroxydases		
Gpx1	Glutathione peroxidase 1	1,110 ± 0,085
Gpx2	Glutathione peroxidase 2	2,071 ± 0,899
Gpx3	Glutathione peroxidase 3	1,580 ± 0,414
Gpx4	Glutathione peroxidase 4	0,012 ± 0,003*
Gpx5	Glutathione peroxidase 5	1,043 ± 0,021
Lpo	Lactoperoxidase	2,042 ± 0,902
Mpo	Myeloperoxidase	2,549 ± 1,318
Hydrolases		
Ephx1	Epoxide hydrolase 1, microsomal	0,732 ± 0,266
Faah	Fatty acid amide hydrolase	0,796 ±0,019*
Fbp1	Fructose-1,6-bisphosphatase 1	2,514 ± 1,297
Kinases		
Hk2	Hexokinase 2	0,012 ± 0,005*
Pklr	Pyruvate kinase, liver and RBC	0,865 ± 0,154
Pkm2	Pyruvate kinase, muscle	0,859 ± 0,031*

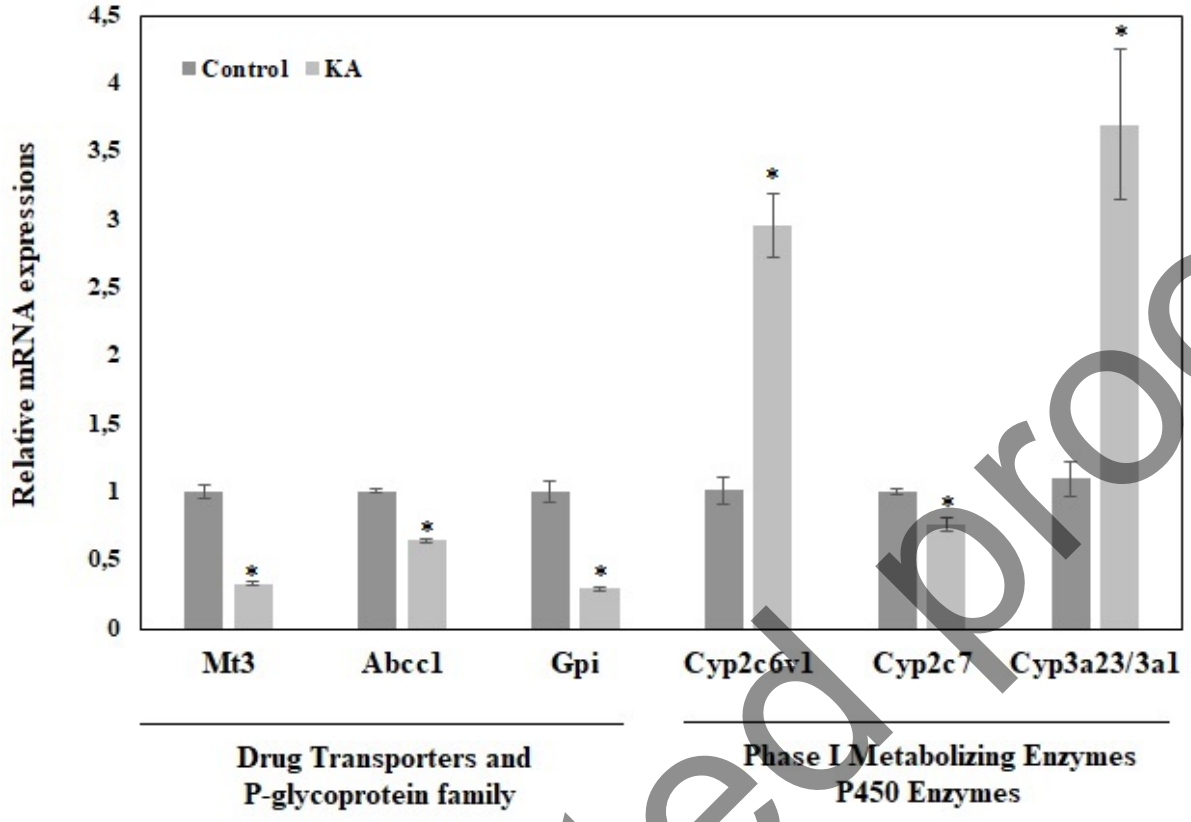

Figure 1. Effects of KA treatment on mRNA expression levels of drug transporters, P-glycoprotein family, and Phase I metabolizing enzymes in hippocampus. Data were expressed as mean ± standard error. $^{*}p<0.05$ vs control in hippocampus (n=6 for each group).

Figure 2. Effects of KA treatment on mRNA expression levels of Phase II metabolizing enzymes in hippocampus. Data were expressed as mean ± standard error. $^{*}p<0.05$ vs control in hippocampus (n=6 for each group).