의학 석사 학위 논문

Alteration of tryptophan metabolites and enzyme activity in aging, exercise and ischemic stroke: study in mouse and human

생쥐 및 인간에서의 노화, 운동 및 허혈성 뇌경색에 따른 트립토판 대사체 및 대사효소 활성 변화에 대한 연구

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Alteration of tryptophan metabolites and enzyme activity in aging, exercise and ischemic stroke: study in mouse and human

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이 논문을 의학석사 학위논문으로 제출함.

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Abstract

Tryptophan is metabolized into metabolites which have neuroprotective or neurotoxic properties. Alteration of the activity of enzymes which mediate tryptophan metabolism pathway is known in various neurological disorders. We investigated tryptophan metabolite level and enzyme activity in mouse groups with different age, and in groups administrated with voluntary chronic aerobic exercise by wheel running. Focal cerebral ischemia model induced by middle cerebral artery occlusion were used to see changes in acute and chronic cerebral ischemia. For human study, serum tryptophan metabolite level and enzyme activity were measured from 55 ischemic stroke patients and 28 control subjects. Demographic data, clinical data and brain MRI findings were retrospectively reviewed. In the animal study, serum kynurenic acid level, kynurenine aminotransferase (KAT) activity, and KAT/kynurenine 3-monooxygenase (KMO) ratio decreased according to age. Exercise group showed higher KAT activity, KAT/KMO ratio and lower KMO activity in some age groups. In cerebral ischemia model, indoleamine 2,3-dioxygenase (IDO) activity and KMO activity increased in acute stage, while KAT activity and KAT/KMO ratio decreased. In the human study KAT and KMO activity decreased according to age. Ischemic stroke patients showed higher IDO activity. Higher IDO activity, lower KAT activity, and KAT/KMO ratio were associated with poor functional outcome. Tryptophan metabolites and enzyme activity alter according to aging, exercise

and cerebral infarction. They are a possible marker for aging and ischemic stroke, and future study might reveal their role in disease mechanism and moreover might be a potential target for intervention.

Key words: tryptophan metabolite, kynurenine pathway, aging, ischemic stroke, aerobic exercise

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Introduction

Tryptophan is one of the 20 amino acids which constitutes protein in human, and is well known as a precursor of serotonin and melatonin.[1] While only about 5% of the tryptophan undergo the serotonin pathway, other 95% is metabolized into kynurenine by indoleamine 2,3-dioxygenase (IDO) and enters through the kynurenine pathway which ends with NAD+, kynurenic acid and xanthurenic acid.[2] Kynurenine further metabolize into kynurenic acid by kynurenine aminotransferase (KAT) which is neuroprotective or produces intermediate metabolite 3-hydroxyl(OH)-kynurenine by kynurenine 3-monooxygenase (KMO) which have neurotoxic properties.[2,3]

Alteration of the metabolites and enzyme activity which mediate kynurenine pathway is known in various neurological disorders such as ischemic stroke, Alzheimer's disease, Parkinson's disease, Huntington's disease and multiple sclerosis.[2-4] Recent studies are focusing on their role in disease mechanism and as a potential target of intervention.[2,4,5]

In this study, we intended to look into alteration of tryptophan metabolites and enzyme activity of the kynurenine pathway in mouse according to aging, changes after intervention by aerobic exercise, and alteration in disease model of ischemic stroke. Additionally, small number of ischemic stroke patients and control subjects were enrolled for validation in human and as a pilot study for future studies.

Methods

Animal study; aging and voluntary chronic aerobic exercise effect

Newborn c57bl6 mice were grouped into 5 groups (n=6 in each group) and were sacrificed at different ages; 6 weeks, 3 months, 6 months, 8 months and 10 months. To evaluate exercise effect, mice in age of 2, 5 and 7 month (young, middle, old age group respectively) were assigned into exercise group (n=5 in each group). Mice in exercise group were exposed to voluntary chronic aerobic exercise for one month by providing a running wheel in the cage.[6,7] For control group, mice with the same age were assigned (n=9 in each group), without running wheel in the cage. In each mouse, blood sample was taken at the time of sacrifice for tryptophan metabolite level quantification. All the protocols for animal study were approved by the Institutional Animal Care and Use Committee of Seoul National University Clinical Research Institute.

Animal study; ischemic stroke model

In a separate group of young age c57bl6 mice, focal cerebral ischemia was induced by intraluminal filamentous occlusion of the middle cerebral artery. The procedures were performed according to methods which were previously described.[8,9] In brief, after intraperitoneal injection of 1%

ketamine (30 mg/kg) and xylazine hydrochloride (4 mg/kg), the left common carotid artery was exposed at its bifurcation by a midline cervical incision. The branches from the external carotid artery (ECA) were then coagulated and the pterygopalatine artery was ligated with a 4-0 silk suture. The ECA was then transected and a 3-0 nylon monofilament suture with tip rounded by heating, was inserted into the ECA stump. To occlude the origins of the MCA and proximal anterior cerebral artery, the suture was advanced into the ICA 15 mm beyond the ICA–pterygopalatine artery bifurcation. The suture was then secured in place with a ligature and the wound closed. After 90 minutes, the reperfusion of MCA was initiated by removing the filament. Ischemic stroke group were further divided into acute and chronic ischemic stroke group. Mice in acute ischemic stroke group (n=5) were sacrificed 24 hours after ischemia induction while chronic ischemic stroke group were sacrificed after 1 month (n=4). Serum tryptophan metabolite level was checked at the time of sacrifice and compared with the identical young age control group mentioned in the above section.

Human pilot study

For human pilot study, serum sample from 55 ischemic stroke patients who admitted to Seoul National University Hospital between March 2011 and July 2012 were acquired. All the patients experienced ischemic

stroke event within 6 months before sampling date. Otherwise, other group of 17 patients who admitted during the same period for because of mild neurological symptoms (headache, dizziness or evaluation for healthcare), and 11 healthy volunteers in their age of 20s or 30s were assigned as control group and serum sample of each patient was taken. Except healthy volunteers, demographic and clinical information were gathered at admission, including age, sex, vascular risk factors (history of hypertension, diabetes mellitus, dyslipidemia, smoking history, heart disease, and previous stroke history) from all admitted patients. Laboratory data was also acquired at the time of admission. Brain MRI was also performed in all admitted patients. Patients who admitted within 1 week after stroke onset were categorized as acute ischemic stroke group. In these patients, National Institutes of Health Stroke Scale (NIHSS) scores at admission, and modified Rankin scale scores (mRS) at discharge were also obtained. Ischemic stroke volume was measured in diffusion weighted image (DWI) sequence according to following ABC/2 method. The largest diameter (A) of the infarct and its largest perpendicular diameter (B) were measured. The third, vertical diameter (C) was determined by summing the thicknesses of the slices in which the lesion was visible. Infarct volume was calculated according to the formula $0.5 \times A \times B \times C$.[10] Data collection and blood sampling were performed in compliance with the regulations of the local institutional review board.

Tryptophan metabolite measurement

Concentrations of tryptophan and its metabolites (kynurenine, kynurenic acid, and 3-OH-kynurenine) in serum were determined with a modified method.[11,12] Briefly, samples were prepared by protein precipitation with methanol. Tryptophan methyl ester was used as an internal standard for the quantification of tryptophan and three metabolites. The quantification was carried out with LC-MS/MS system using an Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, CA, USA) coupled to an Applied Biosystems API4000 triple quadrupole mass spectrometer (AB Sciex, Framingham, MA, USA). Chromatographic separation was conducted on a Synergi Polar-RP (Phenomenex Inc., Torrance, CA, USA) with a mobile phase consisting of 5 mM ammonium formate in distilled water and 0.1% formic acid in methanol. The intra- and inter-day accuracies of this method were from 99.76% to 106.8%, and the intra- and inter-day precisions were <5.4% through the measurements.

To estimate enzyme activity involved in tryptophan metabolism pathway, conversion ratio between two metabolites were used. Activity of IDO was estimated by calculating the ratio of serum kynurenine and tryptophan level (kynurenine level over tryptophan level). By the identical concept, KMO activity was calculated by the ratio of 3OH-kynurenine and kynurenine level (3OH-kynurenine over kynurenine level), and KAT activity by the ratio of kynurenic acid and kynurenine level (kynurenic acid over

kynurenine level). Ratio of KAT activity and KMO activity (KAT/KMO ratio, KAT activity over KMO activity) was also calculated.

Statistical analysis

SPSS for Windows (version 18.0; SPSS, Chicago, IL) was used for statistical analysis. Mann-Whitney U-test and Kurskal-Wallis test were used for comparison of tryptophan metabolites and other interval variables. Chi-square tests were used for dichotomous or nominal variables. Infarct volume measured by NIHSS and mRS scores were dichotomized as follows: an NIHSS score of 4 or more, and less than 4 and mRS score 2 or more and less than 2 considering remained functional deficit respectively. Selected variables showing $P < 0.05$ in bivariate analysis to determine variables associated with discharge mRS were adjusted in the multiple logistic regression model to validate the association with functional outcomes. Statistical significance was considered at $P < 0.05$.

Results

Animal study; tryptophan metabolite level and enzyme activity in aging

Among the mice which were sacrificed at different age groups, there were differences in some serum tryptophan metabolite levels between age groups (Figure 1). Tryptophan, kynurenine and kynurenic acid level showed statistically significant difference. These metabolites seemed to have much lower level in older groups. However, 3-OH-kynurenine level did not show any significant difference between age groups. For enzyme activity, IDO activity showed significance in metabolite level difference, which showed lowest level at 6 month. In addition, KAT activity and KAT/KMO ratio showed much lower level in older groups than in young groups with statistical significance. However, differences in KMO activity did not reach statistical significance.

Animal study; tryptophan metabolite level and enzyme activity in voluntary chronic aerobic exercise and focal cerebral ischemia model

Alteration of tryptophan metabolite and enzyme activity was observed according to voluntary chronic aerobic exercise (Figure 2). Tryptophan and kynurenine level were higher compared to the control group

in old age group and middle age group respectively. 3-OH-kynurenine was lowered in the old age group, while kynurenic acid was elevated in the middle and old age groups. Among the enzyme activities, IDO activity was elevated only in the middle age group, and KMO activity was decreased in the young and old age group. KAT activity and KAT/KMO ratio were elevated in the middle and old age group.

In ischemic stroke models (Figure 3), lower tryptophan and higher kynurenine level were shown in acute cerebral ischemia group. IDO activity was higher in the acute stroke group, while KAT activity and KAT/KMO ratio were lower. 3-OH-kynurenine and kynurenic acid level were not measured in two mice of the chronic cerebral ischemia group because of insufficient serum sample amount and there were no significant difference comparing with other two groups.

Human pilot study; tryptophan metabolite level and enzyme activity in aging

In the whole human subjects, serum kynurenine level and IDO activity were higher according to advanced age groups, while KAT and KMO activity were lower in advanced age groups (Table 1). Analysis among ischemic stroke patients showed difference between age groups only in kynurenine level. Difference of IDO and KAT activity were close to

significance (supplemental table 1). Subjects in the control group showed difference between age groups in kynurenine level, IDO, KAT and KMO activity (supplemental table 2).

Human pilot study; comparison between stroke patients and control group

Characteristics of ischemic stroke patients compared with the control group are presented in table 2. Diabetes (41. 8% vs. 5.9 %, $P = 0.007$), fasting blood glucose level (116.31±36.83 mg·dL^{-1} vs. 91.56±9.79 mg·dL^{-1}, $P = 0.003$), HbA1c (6.41±1.39 % vs. 5.67±0.26 %, $P = 0.032$), white blood cell count (7689.64±2508.22 mL^{-1} vs. 6632.35±3257.08 mL^{-1}, $P = 0.013$) and C-reactive protein (0.39±0.82 mg·L^{-1} vs. 0.13±0.27 mg·L^{-1}, $P = 0.002$) were significantly higher in the ischemic stroke group. Serum level of tryptophan metabolites and enzyme activity in ischemic stroke group and control group are shown in table 3. After including the healthy volunteer subjects, there was a difference in age (66.02±14.83 years vs. 52.14±20.85 years, $P = 0.006$). Serum tryptophan level and IDO activity were higher in the ischemic stroke patients while other metabolites and enzyme activity did not reveal significant difference.

Human pilot study; tryptophan metabolite level, enzyme activity and functional outcome

In order to look into factors associated with functional outcome in acute ischemic stroke. Acute ischemic stroke patients were dichotomized by mRS lesser than 2 and 2 or more. Higher age (63.00±16.61 years vs. 73.45±11,05 years, $P = 0.034$), larger infarct volume (5.36±6.63 mL vs. 32.45±42.92 mL, $P = 0.008$), larger proportion of patients with initial NIHSS 4 or more (28.6 % vs. 81.8 %, $P = 0.004$) were observed (Table 4). In higher mRS group, higher 3-OH-kynurenine level, IDO activity and lower KAT activity and KAT/KMO ratio were shown (Table 5). For multivariate analysis, covariates of age and initial NIHSS 4 or more were inputted in a logistic regression model. 3-OH-kynurenine level ($\beta = 0.211$, $P = 0.074$), KAT activity ($\beta = -27.598$, $P = 0.066$), KAT/KMO ratio ($\beta = -0.454$, $P = 0.068$) were only close to statistical significance while IDO activity ($\beta = 45.251$, $P = 0.197$) did not reveal significance in this model.

Discussion

Animal data of this study demonstrates that tryptophan metabolites and activity of the enzymes contributing to the kynurenine pathway are altered by aging, exercise and cerebral ischemia. We also presented that changes in tryptophan metabolism pathway occurs in human according to aging and ischemic stroke condition. IDO activity seems to be elevated in aging process and cerebral ischemia, while serum kynurenic acid level and KAT activity, KAT/KMO ratio decreased. Moreover, in acute ischemic stroke patients, serum 3-OH-kynurenine level, IDO activity, KAT activity and KAT/KMO ratio were associated with poor functional outcome at discharge. On the other hand, exercise seemed to effect in the opposite direction by lowering 3-OH-kynurenine level, KMO activity and raising kynurenic acid level, KAT activity and KAT/KMO ratio.

Two products of kynurenine metabolism, 3-OH-kynurenine and kynurenic acid are produced via different metabolic pathways and have distinct effects. 3-OH-kynurenie has toxic effects by promoting the production of oxygen free radicals.[13] In contrast, kynurenic acid is known to have neuroprotective effect by inhibiting NMDA (N-methyl-D-aspartate),[14] AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid),[15] kainite[16] and α7 nicotinic acetylcholine receptors[17] and by potent antioxidant properties.[18] IDO is activated in various inflammatory conditions such as infection,[19] tumor,[20]

atherosclerosis,[21,22] obesity[23] and chronic heart disease[24] and is supposed to have a role of immunosuppression parallel to these conditions.[25] KAT and KMO directly contribute to the production of kynurenic acid and 3-OH-kynurenine and are suggested as a target for pharmacological interventions.[4,5,26] The ratio between KAT and KMO activity (KAT/KMO ratio) implies the balance between neuroprotective and neurotoxic products of the pathway.[3]

In aging, data from several animal studies suggest the role of tryptophan metabolism[27] but there were only few human study limited to IDO activity.[28,29] We demonstrated decreasing trend of kynurenic acid, KAT and KAT/KMO ratio in the animal study (Figure 1) which implies reduced neuroprotective capacity as aging proceeds. Human data (Table 1) shows discrepancy with animal data as increased IDO and decreased KMO activity in older age group, which might have been affected by heterogeneity of the subjects, comorbidities, and drugs which the patients are taking. Exercise is known to accelerate tryptophan metabolism by IDO activation.[30,31] A recent animal study revealed exercise increases skeletal expression of KAT by activation of the PGC-1 α1-PPAR α/δ pathway.[32] In our study, we additionally observed reduced KMO activity which suggests other beneficial mechanisms of exercise by reducing oxidative stress (Figure 2).

Alteration of tryptophan metabolites or enzyme activity in ischemic stroke is reported in human studies. Previous studies demonstrate that IDO

activity is associated with other inflammatory markers such as C-reactive protein and erythrocyte sedimentation rate[33,34] and is also known to be associated with infarct volume and functional outcome.[34.35] Our data is in line with these previous reports as IDO activation is shown in both animal study (Figure 3) and in human subjects (Table 1). Moreover, it seemed to show in acute stroke patients, functional outcome at discharge is associated with 3-OH-kynurenine level, KAT activity and KAT/KMO ratio along with IDO activity (Table 5). Unfortunately it was not possible to prove the association after adjustment of covariates probably because of small number of the subjects. However decreased KAT activity and KAT/KMO ratio were also observed in the animal study (Figure 3) and this might support the plausibility of our findings. Interestingly, in the animal study most of the changes were seen in acute stroke model but human subjects did not show any difference between acute and chronic ischemic stroke patients (supplemental table 3). This might be attributed by variable infarct size and time interval between onset to sampling time in the patients.

Our study has several limitations especially in the human study. Number of the subjects was too small to gain enough statistical power in multivariate analysis. Heterogeneous composition of the control group and lack of clinical and laboratory data of healthy volunteers is also needed to be considered.

Conclusion

In this study, we demonstrated that metabolites and activity of the enzymes involved in tryptophan metabolism are altered by conditions of aging, exercise and cerebral ischemia. These findings imply undergoing neurotoxic, neuroprotective and inflammatory processes occurring in these conditions. In addition, these may possibly indicate the functional outcome after acute ischemic stroke. Future study might reveal their role in disease pathomechanism and moreover be a potential target for intervention.

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Table 1. Tryptophan metabolite level and enzyme activity according to age groups

	≤40 (n=15)	41-60 (n=16)	61-80 (n=46)	80< (n=6)	P-value
Tryptophan ($ng \cdot mL^{-1} \pm SD$)	11998.40±2199.35	11125.56±2655.79	11744.35±1877.75	10226.67±2850.23	0.234
Kynurenine ($ng \cdot mL^{-1} \pm SD$)	293.13±47.09	312.49±119.29	407.29±174.61	413.90±63.22	**<0.001***
3-OH-Kynurenine ($ng \cdot mL^{-1} \pm SD$)	9.65±5.21	7.52±2.13	7.98±2.96	8.99±2.20	0.405
Kynurenic acid ($ng \cdot mL^{-1} \pm SD$)	37.45±8.52	36.84±14.06	37.24±13.98	27.26±5.13	0.254
IDO (± SD)	0.025±0.005	0.030±0.014	0.035±0.014	0.043±0.013	**<0.001***
KAT (± SD)	0.131±0.037	0.129±0.052	0.102±0.052	0.066±0.010	**0.001****
KMO (± SD)	0.034±0.020	0.026±0.011	0.021±0.009	0.022±0.006	**0.029****
KAT/KMO ratio (± SD)	4.924±2.472	5.563±3.174	5.339±3.153	3.262±1.233	0.261

IDO indicates indoleamine 2,3-dioxygenase; KAT, kynurenine aminotransferase; KMO, kynurenine 3-monooxygenase; *$P < 0.1$, **$P < 0.05$

Table 2. Comparison of patient characteristics in ischemic stroke group and control group

	Ischemic stroke (n=55)	Control (n=17)	P-value
Male sex, n (%)	35 (63.6)	7 (41.2)	0.101
Mean age in years (± SD)	66.02±14.83	67.53±9,31	0.984
Concomitant disease, n (%)			
Hypertension	36 (35.1)	10 (58.8)	0.619
Diabetes	23 (41.8)	1 (5.9)	**0.007****
Dyslipidemia	29 (52.7)	6 (35.3)	0.209
Heart disease	19 (34.5)	2 (11.8)	0.125
Smoking	7 (12.7)	0 (0.0)	0.187
Laboratory data			
SBP (mmHg ± SD)	142.24±27.16	130.41±14.82	0.097*
DBP (mmHg ± SD)	80.60±14.84	77.35±7.89	0.399
FBS ($mg \cdot dL^{-1}$ ± SD)	116.31±36.83	91.56±9.79	**0.003****
HbA1c (% ± SD)	6.41±1.39	5.67±0.26	**0.032****
Total Cholesterol ($mg \cdot dL^{-1}$ ± SD)	173.82±45.40	185.53±45.01	0.367
LDL Cholesterol ($mg \cdot dL^{-1}$ ± SD)	105.87±39.49	114.21±29.63	0.313
TG Cholesterol ($mg \cdot dL^{-1}$ ± SD)	120.27±68.56	107.21±41.93	0.984

HDL Cholesterol ($mg \cdot dL^{-1} \pm SD$)	47.29±12.35	52.84±11.51	0.075*
WBC ($mL^{-1} \pm SD$)	7689.64±2508.22	6632.35±3257.08	**0.013****
Fibrinogen ($mg \cdot dL^{-1} \pm SD$)	308.02±73.51	290.62±30.74	0.454
CRP ($mg \cdot L^{-1} \pm SD$)	0.39±0.82	0.13±0.27	**0.002****
Homocysteine ($mg \cdot L^{-1} \pm SD$)	11.33±4.26	11.61±7,29	0.691

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; FBS, fasting blood sugar; LDL, low-density lipoprotein; TG, triglyceride; HDL, high-density lipoprotein; WBC, white blood cell count; CRP, C-reactive protein; *$P < 0.1$, **$P < 0.05$

Table 3. Comparison of tryptophan metabolites and enzyme activity between ischemic stroke group and control group

	Ischemic stroke (n=55)	Control (n=28)	P-value
Male sex, n (%)	35 (63.6)	15 (53.6)	0.376
Mean age in years (± SD)	66.02±14.83	52.14±20.85	**0.006****
Tryptophan ($ng \cdot mL^{-1}$ ± SD)	11214.55±2250.65	12242.32±1903.25	**0.048****
Kynurenine ($ng \cdot mL^{-1}$ ± SD)	386.29±174.48	334.63±76.98	0.171
3-OH-Kynurenine ($ng \cdot mL^{-1}$ ± SD)	8.32±3.54	8.16±2.92	1.000
Kynurenic acid ($ng \cdot mL^{-1}$ ± SD)	36.41±36.17	36.62±10.79	0.762
IDO (± SD)	0.035±0.015	0.027±0.007	**0.020****
KAT (± SD)	0.106±0.052	0.116±0.047	0.272
KMO (± SD)	0.024±0.014	0.026±0.011	0.340
KAT/KMO ratio (± SD)	5.014±1.736	4.980±2.031	0.825

IDO indicates indoleamine 2,3-dioxygenase; KAT, kynurenine aminotransferase; KMO, kynurenine 3-monooxygenase; *$P < 0.1$, **$P < 0.05$

Table 4. Clinical and laboratory characteristics patients with acute ischemic stroke. Bivariate analysis with functional outcome.

	mRS <2 (n=28)	mRS 2≤ (n=11)	P-value
Male sex, n (%)	17 (60.7)	9 (81.8)	0.276
Mean age in years (± SD)	63.00±16.61	73.45±11,05	**0.034****
Concomitant disease, n (%)			
Hypertension	14 (50.0)	9 (81.8)	**0.086***
Diabetes	9 (32.1)	5 (45.5)	0.435
Dyslipidemia	15 (53.6)	6 (54.5)	0.956
Heart disease	10 (35.7)	6 (54.5)	0.282
Smoking	5 (17.9)	1 (1.7)	0.655
Laboratory data			
SBP (mmHg ± SD)	140.43±30.91	146.18±22.73	0.315
DBP (mmHg ± SD)	79.82±16.57	85.82±12.25	0.149
FBS ($mg \cdot dL^{-1}$ ± SD)	115.43±42.38	111.36±24.41	0.747
HbA1c (% ± SD)	6.37±1.78	6.19±0.79	0.656
Total Cholesterol ($mg \cdot dL^{-1}$ ± SD)	179.11±39.73	184.82±65.57	0.818
LDL Cholesterol ($mg \cdot dL^{-1}$ ± SD)	107.75±32.86	117.73±56.38	0.818
TG Cholesterol ($mg \cdot dL^{-1}$ ± SD)	123.21±75.84	107.91±85.30	0.116

HDL Cholesterol (mg·dL^{-1} ± SD)	48.29±14.73	46.45±9.72	0.818
WBC (mL^{-1}± SD)	7383.21±2411.15	8609.09±2890.49	0.221
Fibrinogen (mg·dL^{-1} ± SD)	295.11±52.65	331.91±114.64	0.272
CRP (mg·L^{-1}± SD)	0.23±0.24	0.92±1.64	**0.089***
Homocysteine (mg·L^{-1}± SD)	11.05±3.59	13.10±6,00	0.450
Infarct volume (ml^{3})	5.36±6.63	32.45±42.92	**0.008****
NIHSS≥4, n (%)	8 (28.6)	9 (81.8)	**0.004****

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; FBS, fasting blood sugar; LDL, low-density lipoprotein; TG, triglyceride; HDL, high-density lipoprotein; WBC, white blood cell count; CRP, C-reactive protein; NIHSS, NIH Stroke Scale; *P < 0.1, **P < 0.05

Table 5. Relationship between tryptophan metabolites, enzyme activity and modified Rankin scale at discharge

	mRS <2 (n=28)	mRS 2≤ (n=11)	P-value
Tryptophan ($ng \cdot mL^{-1} \pm SD$)	11152.57±1462.78	10984.09±2533.44	0.569
Kynurenine ($ng \cdot mL^{-1} \pm SD$)	343.13±85.12	504.18±317.87	0.072*****
3-OH-Kynurenine ($ng \cdot mL^{-1} \pm SD$)	7.71±4.01	9.73±3.09	**0.015****
Kynurenic acid ($ng \cdot mL^{-1} \pm SD$)	40.57±14.50	32.51±12.73	0.102
IDO (± SD)	0.031±0.010	0.046±0.023	**0.024****
KAT (± SD)	0.125±0.054	0.078±0.039	**0.009****
KMO (± SD)	0.024±0.016	0.023±0.012	0.988
KAT/KMO ratio (± SD)	6.350±3.891	3.735±2.169	**0.010****

IDO indicates indoleamine 2,3-dioxygenase; KAT, kynurenine aminotransferase; KMO, kynurenine 3-monooxygenase; **P* < 0.1, ***P* < 0.05

Supplemental table 1. Tryptophan metabolite level and enzyme activity according to age (Ischemic stroke patients)

	<40 (n=4)	41-60 (n=13)	61-80 (n=33)	80< (n=5)	P-value
Tryptophan ($ng \cdot mL^{-1} \pm SD$)	10981.50±3579.76	10968.69±2733.26	11451.21±1777.96	10478±311.31	0.435
Kynurenine ($ng \cdot mL^{-1} \pm SD$)	291.60±35.76	325.25±128.31	415.10±202.27	430.62±53.84	**0.009****
3-OH-Kynurenine ($ng \cdot mL^{-1} \pm SD$)	11.49±9.18	7.74±2.25	8.07±3.02	8.91±2.45	0.850
Kynurenic acid ($ng \cdot mL^{-1} \pm SD$)	37.80±10.18	35.59±15.15	37.76±14.55	28.49±4.65	0.588
IDO (± SD)	0.028±0.005	0.032±0.015	0.037±0.016	0.044±0.014	0.078*
KAT (± SD)	0.129±0.033	0.120±0.052	0.104±0.055	0.067±0.011	0.074*
KMO (±SD)	0.041±0.036	0.026±0.012	0.021±0.010	0.024±0.014	0.298
KAT/KMO ratio (± SD)	4.912±2.734	5.305±3.411	5.536±3.614	3.462±1.264	0.549

IDO indicates indoleamine 2,3-dioxygenase; KAT, kynurenine aminotransferase; KMO, kynurenine 3-monooxygenase; *$P < 0.1$, **$P < 0.05$

Supplemental table 2. Tryptophan metabolite level and enzyme activity according to age (Control group)

	60< (n=14)	60≥ (n=14)	P-value
Tryptophan ($ng \cdot mL^{-1} \pm SD$)	12237.07±2129.97	11105.05±1728.17	0.769
Kynurenine ($ng \cdot mL^{-1} \pm SD$)	383.38±67.21	285.87±51.69	**0.001>****
3-OH-Kynurenine ($ng \cdot mL^{-1} \pm SD$)	7.87±2.80	8.46±3.12	0.603
Kynurenic acid ($ng \cdot mL^{-1} \pm SD$)	34.86±12.98	38.38±8.16	0.150
IDO (± SD)	0.032±0.074	0.024±0.048	**0.001****
KAT (± SD)	0.094±0.042	0.139±0.041	**0.003****
KMO (± SD)	0.021±0.008	0.030±0.011	**0.035****
KAT/KMO ratio (± SD)	4.656±1.550	5.305±3.411	0.603

IDO indicates indoleamine 2,3-dioxygenase; KAT, kynurenine aminotransferase; KMO, kynurenine 3-monooxygenase; *$P < 0.1$, **$P < 0.05$

Supplemental table 3. Tryptophan metabolites and enzyme activity in patients with different stroke onset

	Acute (n=39)	Chronic (n=17)	Control (n=27)	P-value
Tryptophan ($ng \cdot mL^{-1} \pm SD$)	11105.05±1793.11	11540.76±3069.04	12233.15±1938.88	0.083*
Kynurenine ($ng \cdot mL^{-1} \pm SD$)	388.55±192.69	374.62±123.16	336.80±77.57	0.521
3-OH-Kynurenine ($ng \cdot mL^{-1} \pm SD$)	8.28±3.84	8.41±2.71	8.15±2.98	0.889
Kynurenic acid ($ng \cdot mL^{-1} \pm SD$)	38.29±14.34	33.17±12.59	35.94±10.37	0.470
IDO (± SD)	0.035±0.016	0.035±0.014	0.028±0.007	0.113
KAT (± SD)	0.112±0.054	0.099±0.050	0.113±0.045	0.591
KMO (± SD)	0.024±0.015	0.025±0.011	0.025±0.011	0.646
KAT/KMO ratio (± SD)	5.612±3.663	4.482±2.265	4.925±2.048	0.697

IDO indicates indoleamine 2,3-dioxygenase; KAT, kynurenine aminotransferase; KMO, kynurenine 3-monooxygenase; *$P < 0.1$, **$P < 0.05$

Figure Legends

Figure 1.

Tryptophan metabolites and enzyme activities in mice with different age groups of 6 weeks, 3 month, 6 month, 8 month and 10 month.

Figure 2.

Tryptophan metabolites and enzyme activities in mice with voluntary chronic aerobic exercise for 1 month and mice in control group. *P<0.05, **P<0.01

Figure 3.

Tryptophan metabolites and enzyme activities in mice with induced focal cerebral ischemia. Mice in acute (sacrificed 24 hours after ischemia induction), chronic (sacrificed 1 month after ischemia induction) and control group are compared. *P<0.05, **P<0.01

Figure 1

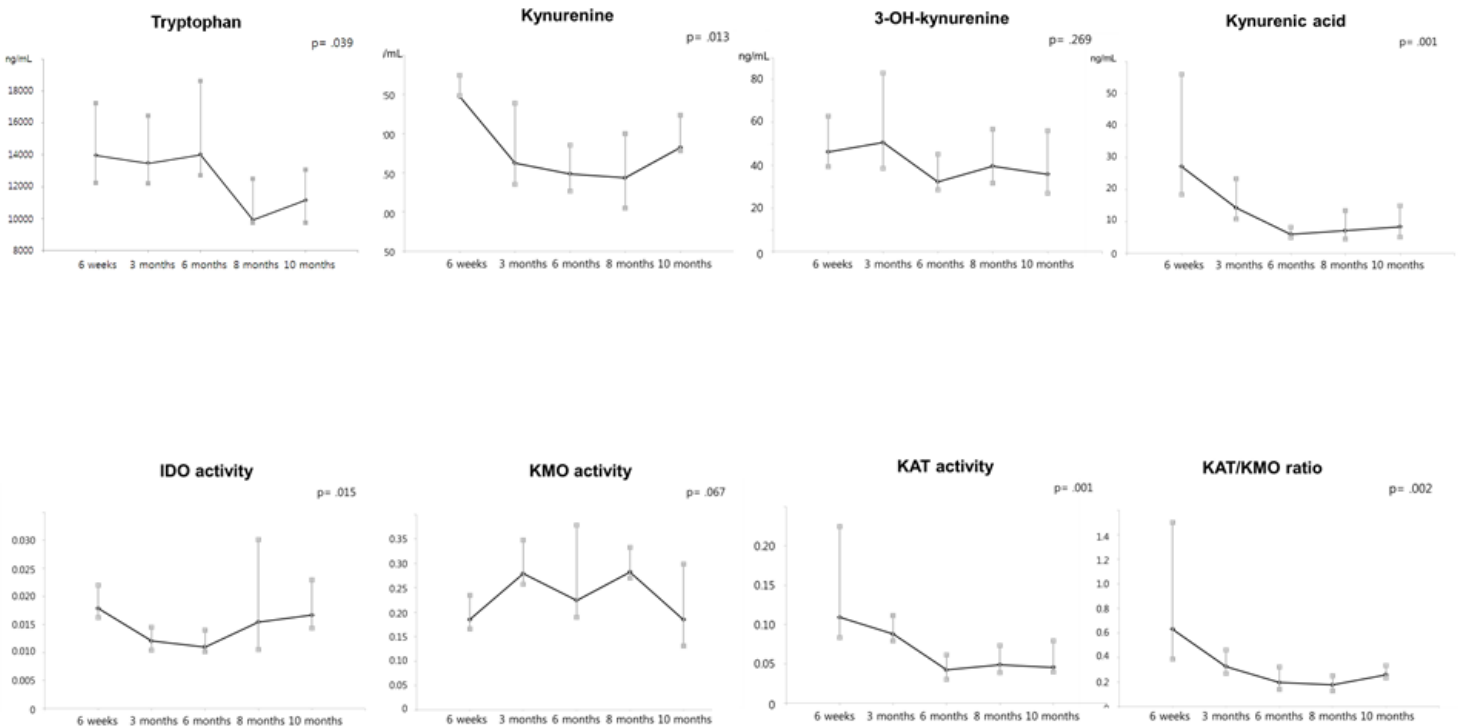

Tryptophan
p= .039
ng/mL
18000
16000
14000
12000
10000
8000
6 weeks 3 months 6 months 8 months 10 months
Kynurenine
p= .013
/mL
50
00
50
6 weeks 3 months 6 months 8 months 10 months
3-OH-kynurenine
p= .269
ng/mL
80
60
40
20
0
6 weeks 3 months 6 months 8 months 10 months
Kynurenic acid
p= .001
ng/mL
50
40
30
20
10
0
6 weeks 3 months 6 months 8 months 10 months
IDO activity
p= .015
0.030
0.025
0.020
0.015
0.010
0.005
0
6 weeks 3 months 6 months 8 months 10 months
KMO activity
p= .067
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
6 weeks 3 months 6 months 8 months 10 months
KAT activity
p= .001
0.20
0.15
0.10
0.05
0
6 weeks 3 months 6 months 8 months 10 months
KAT/KMO ratio
p= .002
1.4
1.2
1.0
0.8
0.6
0.4
0.2
6 weeks 3 months 6 months 8 months 10 months

Figure 2

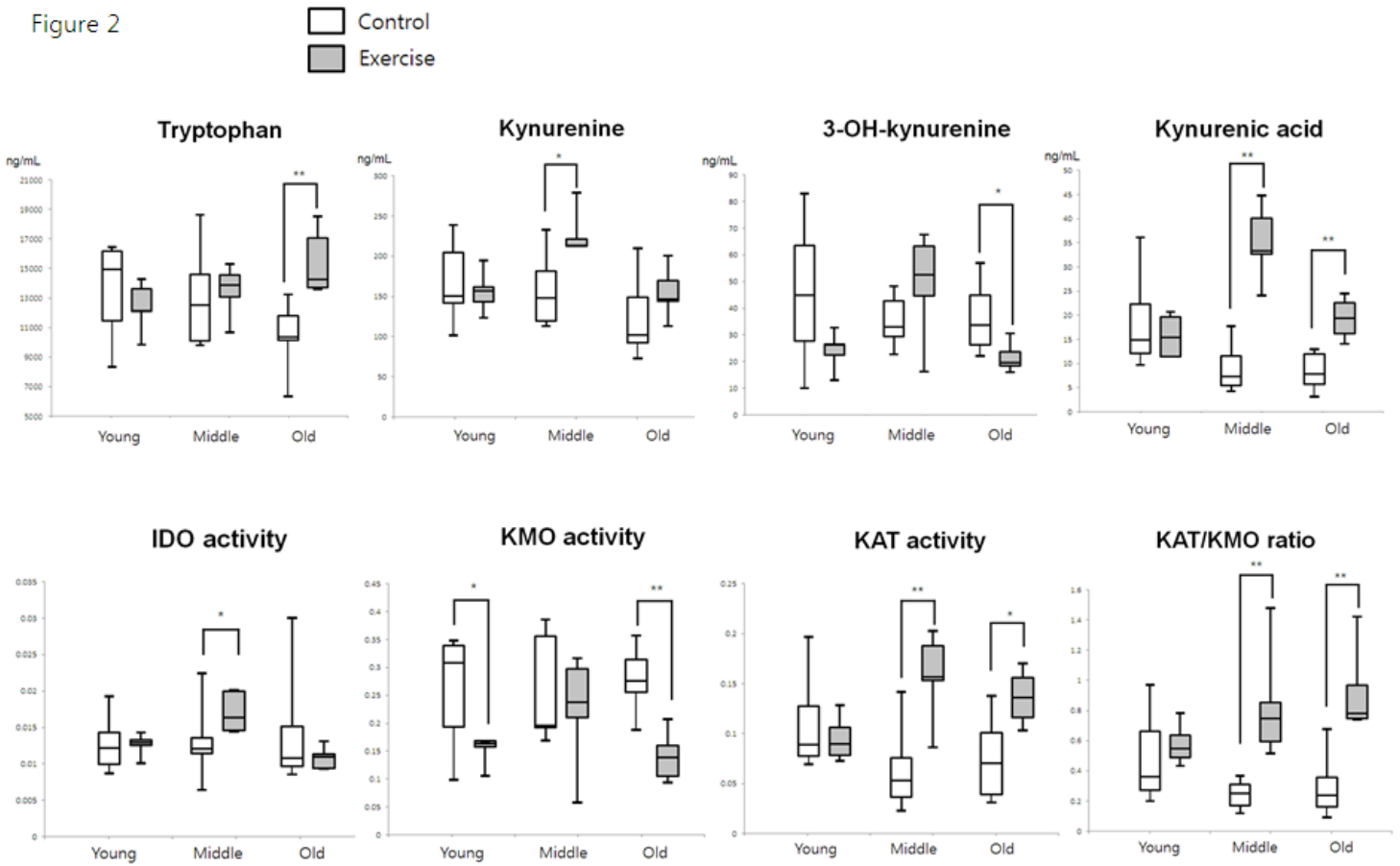
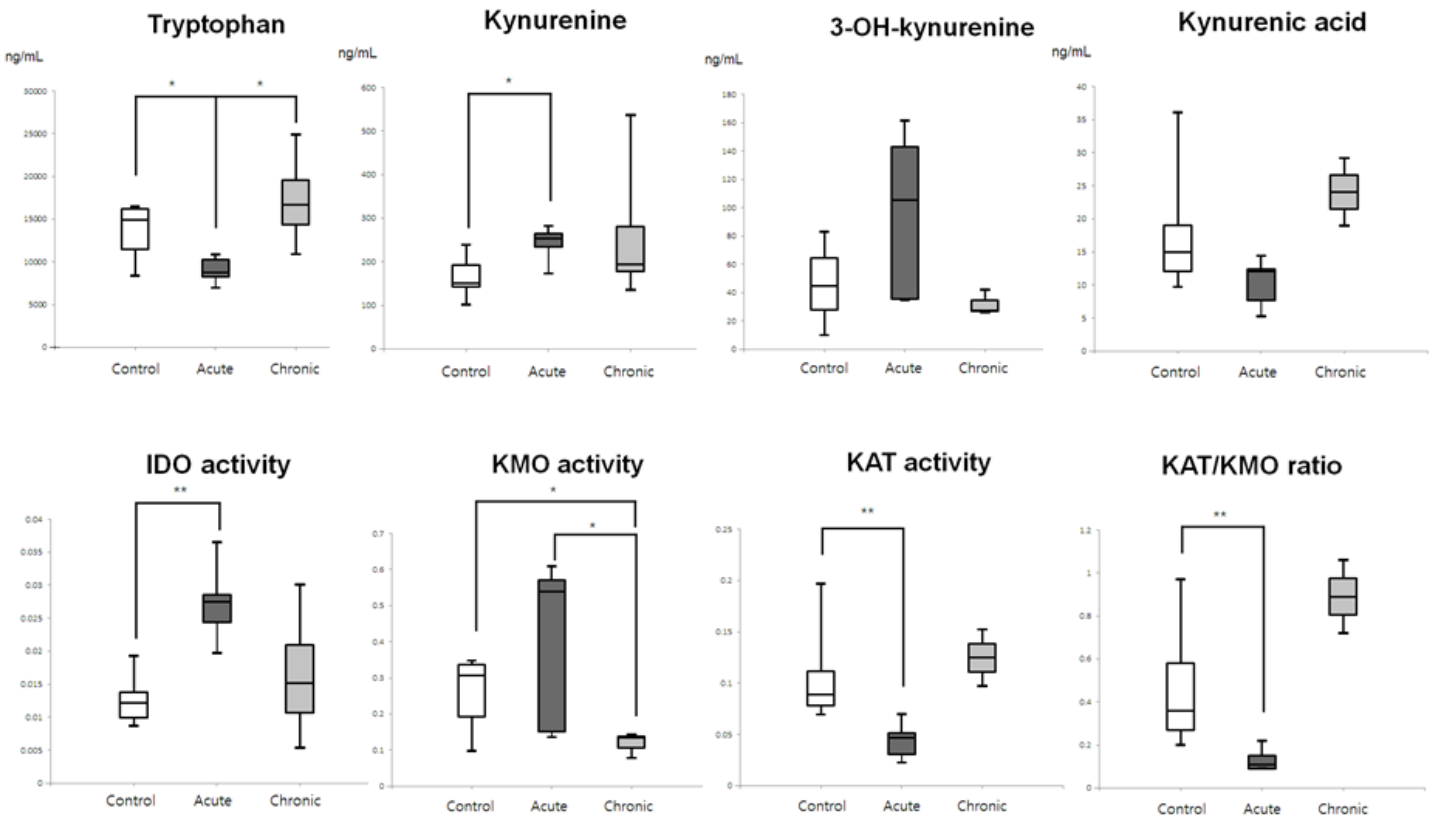

Control
Exercise
Tryptophan
Kynurenine
3-OH-kynurenine
Kynurenic acid
ng/mL
Young
Middle
Old
IDO activity
KMO activity
KAT activity
KAT/KMO ratio

Figure 3


Tryptophan
ng/mL
Control
Acute
Chronic
Kynurenine
ng/mL
Control
Acute
Chronic
3-OH-kynurenine
ng/mL
Control
Acute
Chronic
Kynurenic acid
ng/mL
Control
Acute
Chronic
IDO activity
Control
Acute
Chronic
KMO activity
Control
Acute
Chronic
KAT activity
Control
Acute
Chronic
KAT/KMO ratio
Control
Acute
Chronic

국문초록

트립토판은 체내에서 신경보호효과 또는 신경독성을 나타내는 다양한 대사체로 대사된다. 트립토판 대사경로에 작용하는 대사효소의 활성변화는 다양한 신경계 질환에서 알려져 있다. 본 연구에서는 다른 연령군 및 자발 유산소 운동에서 생쥐의 대사체 농도 및 효소활성 변화를 살펴보았다. 또한 뇌허혈에서의 변화를 보기 위해 중뇌동맥폐쇄를 통한 국소 뇌허혈 모델을 사용하였다. 임상연구를 위해 55 명의 뇌경색 환자와 28 명의 대조군에서의 대사체 농도를 측정하였고, 역학, 임상정보와 뇌 MRI 정보를 수집하였다. 동물실험에서 kynurenic acid 농도, kynurenine aminotransferase (KAT) 활성, 그리고 KAT/kynurenine 3-monooxygenase (KMO)비는 고령군에서 보다 낮았다. 자발 유산소 운동을 시행한 군에서 보다 높은 KAT 활성과 낮은 KMO 활성이 일부 군에서 관찰되었다. 뇌허혈 모델에서, indoleamine 2,3-dioxygenase (IDO)활성과 KMO 활성이 급성기에서 증가하였으나, KAT 활성과 KAT/KMO 비는 감소하였다. 임상연구결과, KAT 와 KMO 활성은 연령에 따라 감소하였고, 뇌경색 환자들은 대조군에 비해 높은 IDO 활성을 보였으며 높은 IDO 활성과 낮은 KAT 활성, KAT/KMO 비는 나쁜 예후와 연관이

있었다. 본 연구를 통해 트립토판 대사체와 대사효소활성은 노화, 운동 및 뇌허혈에 따라 변화하는 것을 확인하였다. 이는 향후 노화 및 뇌경색에서의 표지자로써 활용가치가 있으며, 향후 추가 연구를 통해 이의 병태생리학적 역할 규명 및 질병치료에의 활용을 모색해 볼 수 있겠다.

주요어: 트립토판 대사체, 카이뉴레닌 경로, 노화, 뇌경색, 유산소 운동

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