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ORIGINAL RESEARCH

Kynurenic Acid Metabolism in Various Types of Brain Pathology in HIV-1 Infected Patients

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Abstract: Kynurenic acid, an intermediate metabolite of L-kynurenine, is a competitive antagonist of inotropic excitatory amino acid (EAA) receptors as well as a non competitive antagonist of 7 alpha nicotine cholinergic receptors and its involvement in memory deficit and cognition impairment has been suggested. Alterations of kynurenic acid metabolism in the brain after HIV-1 (human immunodeficiency virus type-1) infection have been demonstrated. The present study evaluates the biosynthetic machinery of kynurenic acid e.g. the content of L-kynurenine and kynurenic acid, as well as the activity of enzymes synthesizing kynurenic acid, kynurenine aminotransferase I (KAT I) and kynurenine aminotransferase II (KAT II) in the frontal cortex and cerebellum of HIV-1 infected patients in relation to different types of pathology classified as follows: HIV in brain (HIV); opportunistic infection (OPP); infarction of brain (INF); malignant lymphoma of brain (LY); and glial dystrophy (GD) and of control (CO) subjects. Of all investigated pathologies the most frequent was OPP (65%), followed by HIV (26%), LY, INF, and GD (each 22%, respectively). Further, 68% of HIV-1 patients had bronchopneumonia, the highest incidence of which, at 60%, was seen in the OPP and LY group. Kynurenic acid was increased significantly in the frontal cortex of LY (392% of CO, $P < 0.001$), HIV (231% of CO, $P < 0.01$) and GD (193% of CO, $P < 0.05$), as well as in the cerebellum of GD (261% of CO, $P < 0.01$). A significant increase of L-kynurenine was observed in the frontal cortex of LY (385% of CO, $P < 0.001$) and INF (206% of CO, $P < 0.01$), and in the cerebellum of GD, LY, OPP and HIV (between 177% and 147% of CO). The KAT I activity increased significantly in the frontal cortex of all pathological subgroups, ie OPP = 420% > INF > LY > HIV > GD = 192% of CO. In the cerebellum, too, all pathological subgroups showed marked increase of KAT I activity (OPP = 320% > LY, HIV > GD > INF = 176% of CO). On contrary, the activity of KAT II was moderately, but significantly, higher in the frontal cortex of INF and OPP; in the cerebellum of HIV, OPP and LY it was comparable to the control, while mildly reduced in INF and GD. Interestingly, normal subjects with the diagnosis of bronchopneumonia were characterized by high kynurenic acid metabolism in the brain, too. Correlation analyses between kynurenine parameters revealed association between high ratio KAT I/KAT II and increased kynurenic acid level and lower L-kynurenine in the frontal cortex and cerebellum of HIV and LY subgroups.
The present study revealed a different pattern of alteration of kynurenic acid metabolism in frontal cortex and cerebellum among investigated pathological subgroups of HIV-1 infected patients. Interestingly, a marked enhancement of kynurenic acid metabolism in the brain has been found with occurrence of bronchopneumonia. This finding indicates a notable association between impaired conditions of oxygen availability and enhancement of kynurenic acid formation in the human brain. These observation(s) might have an impact on the understanding of pathological processes in the brain after HIV-1 infection involving the development of neuropsychiatric and neurological symptoms, including memory and cognition impairment.

Keywords: HIV-1 infection, excitatory amino acid, kynurenic acid, kynurenine aminotransferases, neuroprotection, dementia, bronchopneumonia

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Introduction

A disturbance of L-tryptophan metabolism was demonstrated in human immunodeficiency virus type-1 (HIV-1) associated neurological deficits. Some reports have described lowered L-tryptophan, elevated L-kynurenine, quinolinic acid and kynurenic acid in the CSF of HIV-1 patients.[1–5] Quinolinic acid and many others neurotoxins are enhanced in the brain after cerebral HIV-1 infection and likely might contribute to the pathogenesis of neurological impairment.[6–10] Furthermore, after HIV-1 infection, an increase of kynurenic acid levels and an enhancement of kynurenine aminotransferase I (KAT I) and kynurenine aminotransferase II (KAT II) activities, the enzymes synthesizing kynurenic acid, were found in the brain.[11] The ability of kynurenic acid to block excitotoxic neuronal damage caused by neurotoxins and hypoxia/ischemia in animal experiments strongly supports the idea that kynurenic acid might function as a neuroinhibitory/modulatory metabolite in the human brain.[3,12,13] On the other hand, increased kynurenic levels in the brain of Alzheimer's patients[14] or during aging process[15] was suggested as a significant event contributing to cognitive impairment and memory deficit. This assumption was supported by the observation that the anti-dementia drug Cerebrolysin lowered kynurenic acid synthesis, at least in an in vitro human study.[16] An animal experimental study also provides significant evidence that increased levels of kynurenic acid in the brain enhances memory impairment.[17,18]

After HIV-1 infection neuronal cell death accompanied by astrocytosis takes place.[10,19–21,34] Major characteristics are widespread reactive astrocytosis, myelin pallor, and infiltration of monocytic cells, including blood-derived macrophages, resident microglia, and multinucleated giant cells. Most of these cells expressed very high metabolism (turnover) accompanied by complex tissue damage. After HIV-1 infection, a complex brain damage takes place described by different types of pathology thus HIV-1 in brain as found by anti-HIV immunocytochemistry (HIV), opportunistic infection (OPP), infarction of brain (INF), malignant lymphoma of brain (LY), and glial dystrophy (GD).[20] Beside the different brain pathology, HIV-1 infected subjects often develop pneumonia or bronchopneumonia and a high mortality has been observed.[21] The aim of the present study was to evaluate the alterations of kynurenic acid metabolism in relation to different types of brain pathology in HIV-1 infected patients and possible involvement in cognitive impairment and dementia is discussed. A part of this study was published in abstract form.[22]

Materials and Methods

Chemicals

L-kynurenine, kynurenic acid, pyruvate, pyridoxal-5′-phosphate and 2-amino-2-methyl-1-propanol (AMPOL) were purchased from Sigma. All other chemicals used were of the highest commercially available purity.

Brain tissue

Post mortem human brain samples of frontal cortex and cerebellum had been stored frozen at the Institute of Neurology, University of Vienna. Patients were clinically assessed and their diagnoses were based on clinical history and neuro-histopathological[20] examination eg, 23 patients infected with HIV-1 aged 40.1 ± 3.0 years, and 16 controls (CO) aged 58.2 ± 3.4 years. Ratio male/female was 20/3 for HIV-1 patients, and 9/7 for CO. Post mortem time was 25.4 ± 4.4 hrs in HIV-1 and 10.1 ± 1.6 hrs in CO. In addition, as a separate group of 3 controls patients, aged 51.0 ± 8.3 years, with bronchopneumonia were analyzed in this study (post mortem time was 9.0 ± 0.6 hrs; ratio male/female was 2/1). Brain samples were stored at −70 °C before analysis. None of the patients was treated with anti-retroviral drugs. Samples were always taken from areas without gross lesions.

Pathology

HIV-1 infection leads to a broad spectrum of pathology types in the brain and these were classified as follows: OPP (n = 15); HIV (n = 6); LY (n = 5); INF (n = 5); and GD (n = 5) (Table 1). The occurrence of bronchopneumonia and tuberculosis in pathological subgroups is shown in the Table 2.

Tissue preparation

Brain samples were homogenized in an ice bath in 6 volumes (wt/vol) of 5 mM Tris-acetate buffer pH 7.4 containing 50 µM pyridoxal-5′-phosphate and 10 mM mercaptoethanol. Obtained homogenate was divided in two parts one for L-kynurenine and kynurenic acid determinations, and the other for KATs activities measurements.

Table 1. Occurrence of pathologies after HIV-1 infection.

	OPP	HIV	LY	INF	GD
Ratio of pathology cases/total patient numbers infected with HIV-1; expressed in %	15/23 (65%)	6/23 (26%)	5/23 (22%)	5/23 (22%)	5/23 (22%)

Abbreviations: OPP, Opportunistic infection; HIV, HIV in brain as seen by immunocytochemistry/HIVE, HIVL; LY, Malignant lymphoma; GD, Glial dystrophy; INF, Infarction of brain.

Determination of kynurenic acid and L-kynurenine

Homogenate was mixed with 0.2 M HCl (vol/vol) and centrifuged (20 min, 15,000 g). Obtained supernatant was applied to a Dowex 50 W cation exchange column prewashed with 0.1 M HCl. Subsequently, the column was washed with 1 mL 0.1 M HCl and 1 mL distilled water, and kynurenic acid was eluted with 2 mL distilled water,[23] while L-kynurenine was eluted with 2 mL of 1 M NH_4OH.[24] Kynurenic acid was determined by HPLC coupled to fluorescence detection system as previously reported.[25] The HPLC system used for analysis of kynurenic acid consisted of the following: a pump (Shimadzu, LC-6A), a fluorescence detector (Shimadzu, RF-535) set at an excitation wave length of 340 nm and an emission wavelength of 398 nm, and a Shimadzu C-R5A Chromatopac Integrator. The mobile phase (isocratic system) consisted of 50 mM sodium acetate, 250 mM zinc acetate and 4% acetonitrile, pH 6.2, and was pumped through a 10 cm × 0.4 cm column (HR-80, C-18, particle size 3 µM, InChrom, Austria) at a flow rate of 1.0 mL/min, run at room temperature (23 °C). L-kynurenine was quantitated by HPLC coupled with UV detector at 365 nm. Mobile phase contained 0.1 M ammonium acetate, 0.1 M acetic acid, and 2% acetonitrile.[26]

Determination of KAT I and KAT II activities

KAT I and KAT II were determined by the method of Mason 1954[27] followed by modification.[14,28] Briefly, the reaction mixture contained the homogenate, 2 µM L-kynurenine, 1 mM pyruvate, 70 µM pyridoxal 5′-phosphate and 150 mM AMPOL buffer, pH 9.6 (for KAT I) or 150 mM Tris-acetate buffer, pH 7.4 (for KAT II), in a total volume of 0.2 mL. After the incubation for 16 hrs at 37 °C (linearity of enzyme activity up to 18 hrs was ascertained in pilot experiments) the reaction was terminated by the addition of 10 µL of 50% TCA. Subsequently, 1 mL of 0.1 M HCl was added and denatured protein was removed by 10 min centrifugation. The supernatant was applied to a Dowex 50 W cation exchange column. Eluted kynurenic acid from the column was determined by HPLC method, as described above. The blanks were obtained by using tissue which has been heat inactivated for 30 min in a boiling water bath.

Protein determination

Protein was measured according to the method of Bradford (1976)[29] using a commercially available kit (BIO-RAD) and bovine serum albumin as a standard.

Statistics

All data are presented as the means ± standard error of the mean. For statistical analyses, one-way ANOVA analysis of variance and a Student's *t*-test were applied. Each sample was determined in duplicate or triplicate. The level for statistical significance was $P < 0.05$. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$

Table 2. Occurrence of bronchopneumonia (BR) and tuberculosis (TB) in different pathological group of patients after HIV-1 infection.

Ratio	OPP	HIV	LY	INF	GD
Number of pathology cases with BR/ total number of pathology cases; expressed in %	9/15 (60%)	1/6 (17%)	3/5 (60%)	2/5 (40%)	2/5 (40%)
Number of pathology cases with TB/ total number of pathology cases; expressed in %	2/15 (13%)	2/6 (33%)	1/5 (20%)	No case with TB	1/5 (20%)

Abbreviations: OPP, Opportunistic infection; HIV, HIV in brain as seen by immunocytochemistry/HIVE, HIVL; INF, Infarction of brain; LY, Malignant lymphoma; GD, Glial dystrophy; BR, Bronchopneumonia; TB, Tuberculosis.

indicate a significance compared with control group. Linear regression analysis was performed using the least squares methods. Correlation between kynurenine parameters, eg, between kynurenic acid and L-kynurenine or KAT I, KAT II, ratio KAT I/KAT II, and combined KAT I and KAT II was analyzed.

Results

Subgroups of HIV-1 infected patients

Among investigated HIV-1 infected patients, different pathologies were detected. The most frequent pathology was OPP (65%) followed by HIV (26%) then LY, INF, and GD (each 22%, respectively) (Table 1). Among different pathologies bronchopneumonia was present in all subgroups but frequently present in OPP and LY group (60% each group) and tuberculosis was present in all pathological subgroups, except of INF, but to lesser extent (Table 2).

Kynurenic acid in different pathologies after HIV-1 infection

Kynurenic acid increased significantly in the frontal cortex of LY (392% of CO, $P < 0.001$), INF (247% of CO, $P = 0.056$), HIV (231% of CO, $P < 0.01$) and GD (193% of CO, $P < 0.05$) and in the cerebellum of GD (261% of CO, $P < 0.01$), (Fig. 1). No significant increase of kynurenic acid was found in the frontal cortex of OPP, and in the cerebellum of LY, OPP and INF. One-way analysis of variance (ANOVA) between the groups revealed the means of kynurenic acid statistically different in the frontal cortex (F = 2.4140, $P = 0.050$, one-way ANOVA, Fig. 1) and not statistically different in the cerebellum (F = 1.5210, $P = 0.2050$, one-way ANOVA, Fig. 1).

L-kynurenine in different pathologies after HIV-1 infection

L-kynurenine increased significantly in the frontal cortex of LY (385% of CO, $P = 0.001$) and INF (206% of CO, $P < 0.01$) and in the cerebellum (between 147 and 177% of CO) of all pathologies, except of GD, (Fig. 2). One-way ANOVA between 6 groups revealed the means of L-kynurenine statistically different in the frontal cortex (F = 2.7017, $P = 0.0319$, one-way ANOVA, Fig. 2) and in the cerebellum (F = 2.9019, $P = 0.0233$, one-way ANOVA, Fig. 2).

KAT I activity in different pathologies after HIV-1 infection

KAT I activity was significantly increased in the frontal cortex of all pathological subgroups, ie, OPP = 420% > INF > LY > HIV > GD = 192% of CO, respectively (Fig. 3). In the cerebellum of

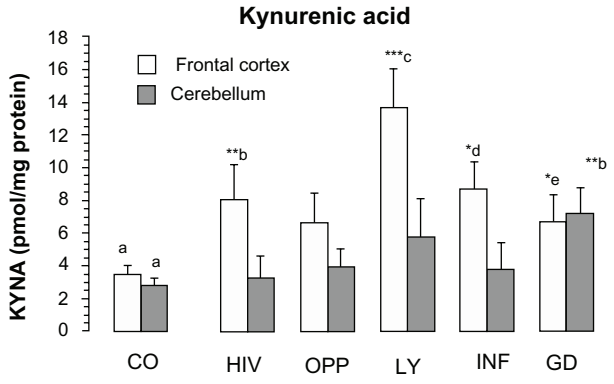

Figure 1. Kynurenic acid level in the frontal cortex and in the cerebellum of different pathological sub-groups of patients after HIV-1 infection.
Notes: Values represent the mean ± SEM of following: Control (CO, n = 16); Pathological subgroups: HIV in brain tissue by immunocytochemistry (HIV, n = 6); opportunistic infection (OPP, n = 15); malignant lymphoma (LY, n = 5); infarction of brain (INF, n = 5) and glial dystrophy (GD, n = 5). Significance: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared with control. Statistical analysis for frontal cortex: a, d and a, e indicate statistical significance at $P < 0.05$; a, b indicates statistical significance at $P < 0.01$; a, c indicates statistical significance at $P < 0.001$. Analysis of variance: F = 2.4140, $P = 0.050$. Statistical analysis for cerebellum: a,b indicates statistical significance at $P < 0.01$. One-way analysis of variance: F = 1.5210, $P = 0.2030$.

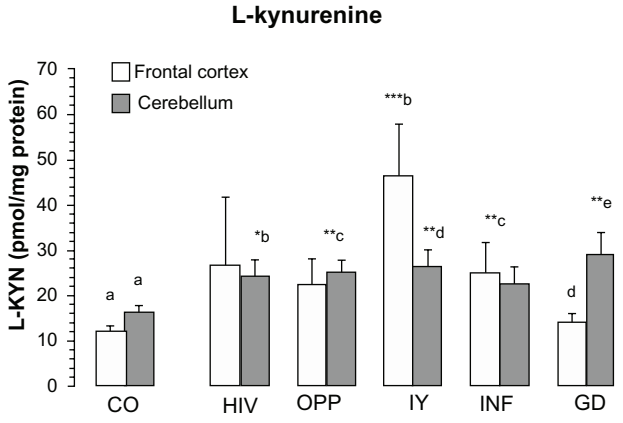

Figure 2. L-kynurenine content in frontal cortex and cerebellum of different pathological subgroups HIV-1 infection.
Notes: Values represent the mean ± SEM: Control (CO, n = 16); Pathological subgroups: HIV in brain tissue by immunocytochemistry (HIV, n = 6); opportunistic infection (OPP, n = 15); malignant lymphoma (LY, n = 5); infarction of brain (INF, n = 5) and glial dystrophy (GD, n = 5). Significance: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared with control. Statistical analysis for frontal cortex: b, d indicate statistical significance at $P < 0.05$; a, c indicates statistical significance at $P < 0.01$; a, b indicates statistical significance at $P < 0.001$, analysis of variance: F = 2.7017, $P = 0.0319$. Statistical analysis for cerebellum: a, b indicates statistical significance at $P < 0.05$; a, c; a, d; a, e indicate statistical significance at $P < 0.01$, one-way analysis of variance: F = 2.9019, $P = 0.0233$.

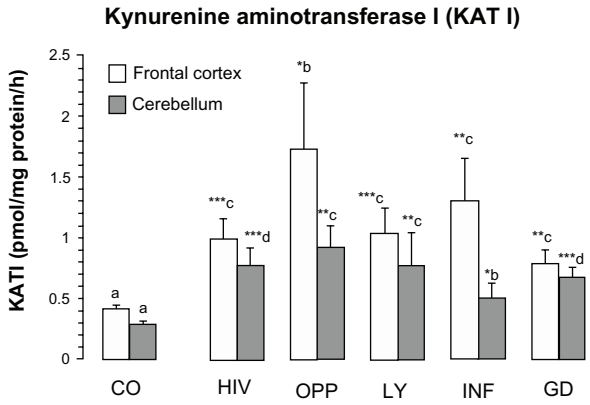

Figure 3. Kynurenine aminotransferase I (KAT I) in the frontal cortex and cerebellum of different pathological subgroups of patients after HIV-1 infection.
Notes: Values represent the mean ± SEM of following groups: Control (CO, n = 16); Pathological subgroups: HIV in brain tissue by immunocytochemistry (HIV, n = 6); opportunistic infection (OPP, n = 15); malignant lymphoma (LY, n = 5); infarction of brain (INF, n = 5) and glial dystrophy (GD, n = 5). Significance: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared with control. Statistical analysis for frontal cortex: a, b indicates statistical significance at $P < 0.05$; a, c indicates statistical significance at $P < 0.001$; analysis of variance: F = 1.9226, $P = 0.1088$. Statistical analysis for cerebellum: a, b indicates statistical significance at $P < 0.05$; a, c indicates statistical significance at $P < 0.01$, a, d indicates statistical significance at $P < 0.001$, one-way analysis of variance, F = 3.2938, $P = 0.0126$.

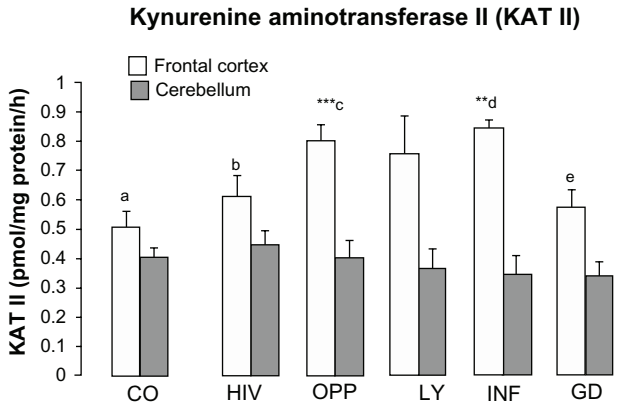

Figure 4. Kynurenine aminotransferase II (KAT II) in the frontal cortex and cerebellum of different pathological subgroups of patients after HIV-1 infection.
Notes: Values represent the mean ± SEM of following groups: Control (CO, n = 16); Pathological subgroups: HIV in brain tissue by immunocytochemistry (HIV, n = 6); opportunistic infection (OPP, n = 15); malignant lymphoma (LY, n = 5); infarction of brain (INF, n = 5) and glial dystrophy (GD, n = 5). Significance: **$P < 0.01$; ***$P < 0.001$ compared with control Statistical analysis for frontal cortex: c, e indicates statistical significance at $P < 0.05$; b, d indicates statistical significance at $P < 0.02$; a, d indicates statistical significance at $P < 0.01$; d, e indicates statistical significance at $P < 0.003$; a, c indicates statistical significance at $P < 0.001$, analysis of variance: F = 4.366, $P = 0.002$. Statistical analysis for cerebellum; one-way analysis of variance, F = 0.3238, $P = 0.8960$.

all pathological subgroups KAT I activity increased (OPP = 320% > LY, HIV > GD > INF = 192% of CO, Fig. 3) significantly, too, one-way ANOVA between 6 groups revealed no statistical difference in the means of KAT I activity in the frontal cortex (F = 1.9226, $P = 0.1088$, one-way ANOVA, Fig. 3), but statistically different in the cerebellum (F = 3.2938, $P = 0.0126$, one-way ANOVA, Fig. 3).

KAT II activities in different pathologies after HIV-1 infection

KAT II activity was moderately but significantly increased in the frontal cortex of INF and OPP, whereas in the cerebellum of HIV, OPP and LY, KAT II activity was comparable to control, while mildly reduced in INF and GD (Fig. 4). One-way ANOVA between 6 groups revealed the means of KAT II activity statistically different in the frontal cortex (F = 4.3664, $P = 0.0025$, one-way ANOVA, Fig. 4), but not statistically different in the cerebellum (F = 0.3238, $P = 0.8961$, one-way ANOVA, Fig. 4).

Kynurenic acid metabolism in the brain of subjects with bronchopneumonia

Three control cases with bronchopneumonia had abnormal L-kynurenine parameters in the brain (Table 3). A marked increase of kynurenic acid level was found in the frontal cortex (383% of CO, $P < 0.01$), but a tendency towards decreased kynurenic acid was observed in the cerebellum. On the contrary to KAT II, KAT I activity was significantly increased in the frontal cortex (877% of CO, $P < 0.01$) and cerebellum (479% of CO; $P < 0.05$), (Table 3).

Correlation between kynurenic acid and L-kynurenine in the frontal cortex and cerebellum

A positive correlation between kynurenic acid and L-kynurenine was found in the frontal cortex of INF group (R = 0.9676, F = 44.0554, $P = 0.0069$, one-way ANOVA, Fig. 5) and no significant correlation between kynurenic acid and L-kynurenine was seen in the frontal cortex of remaining groups ie, HIV (R = –0.5986, F = 2.2339, $P = 0.2093$); OPP (R = –0.0921, F = 0.1112, $P = 0.7442$); LY (R = 0.4795, F = 0.8956, $P = 0.4138$); GD (R = 0.6270, F = 1.9436, $P = 0.2576$) and of CO (R = 0.0688, F = 3.8851, $P = 0.0688$, one-way ANOVA, Fig. 6). Also, no significant correlation of kynurenic acid and L-kynurenine was seen in the cerebellum of CO (R = –0.1609, F = 0.3723, $P = 0.5515$); HIV (R = 0.1289, F = 0.0068, $P = 0.8076$); OPP (R = 0.0037,

Table 3. Kynurenic acid levels and activity of KAT I and KAT II in the frontal cortex and cerebellum of 3 patients with bronchopneumonia.

Brain region	KYNA	KAT I	KAT II
Frontal cortex	13.383 ± 2.290	3.603 ± 1.226	0.511 ± 0.028
expressed in %	(383% of CO)**	(877% of CO)**	(101% of CO)
Cerebellum	2.117 ± 0.005	1.371 ± 0.680	0.513 ± 0.168
expressed in %	(76% of CO)	(479% of CO)*	(127% of CO)

Notes: Values represent the mean ± SEM. Significance: *$P < 0.05$; **$P < 0.01$ compared with control (CO). Values of CO parameters in the frontal cortex (n = 16) and cerebellum (n = 16) for KYNA were 3.49 ± 0.55 and 2.77 ± 0.63 (pmol/mg protein), respectively; for KAT I were 0.41 ± 0.04 and 0.29 ± 0.03 (pmol/mg protein/h), respectively; for KAT II were 0.51 ± 0.06 and 0.40 ± 0.06 (pmol/mg protein/h), respectively.

F = 0.0018, $P = 0.9896$); LY (R = 0.2975, F = 0.2912, $P = 0.6269$); INF (R = 0.3137, F = 0.3275, $P = 0.6072$) and GD (R = 0.4508, F = 0.7653, $P = 0.4461$), using one-way ANOVA.

Correlation between kynurenic acid and KAT I in the frontal cortex and cerebellum

No significant correlation was found between kynurenic acid level and KAT I activity in the frontal cortex of CO (R = 0.0158, F = 0.0035, $P = 0.9536$, one-way ANOVA, Fig. 7A) and of pathological groups ie, HIV (R = –0.1794, F = 0.1331, $P = 0.7338$); OPP (R = –0.0940, F = 0.1159, $P = 0.7389$), LY (R = 0.0233, F = 0.0016, $P = 0.9703$); INF (R = –0.0789, F = 0.0188, $P = 0.8997$) and GD (R = –0.4018, F = 0.5025, $P = 0.5025$) using one-way ANOVA. No significant correlation was seen between kynurenic acid level and KAT I activity in the cerebellum of HIV (R = 0.2786, F = 0.3366, $P = 0.5929$); OPP (R = –0.1728, F = 0.4002, $P = 0.5379$); LY (R = 0.6836, F = 2.6318, $P = 0.2032$); INF (R = –0.4050, F = 0.5888, $P = 0.4987$) and GD (R = –0.0488, F = 0.0072, $P = 0.9379$) except for significant correlation between kynurenic acid and KAT I in the cerebellum of CO (R = 0.5171, F = 6.1106, $P = 0.0402$, one-way ANOVA, Fig. 7B).

Correlation between kynurenic acid and KAT II in the frontal cortex and cerebellum

No significant correlation was observed between kynurenic acid level and KAT II activity in the frontal cortex of CO (R = 0.0103, F = 0.1501, $P = 0.7042$) and of all pathological subgroups ie, HIV (R = –0.2036, F = 0.1730, $P = 0.6988$), OPP (R = 0.2529, F = 0.8887, $P = 0.3630$); LY (R = 0.6945, F = 2.7952, $P = 0.1931$); INF (R = 0.3462, F = 0.4085, $P = 0.5682$) and GD (R = 0.0745, F = 0.0167, $P = 0.9052$) using one-way ANOVA. In addition, no significant correlation was found between kynurenic acid level and KAT II activity in the cerebellum of CO (R = 0.1392, F = 0.2767, $P = 0.6071$)

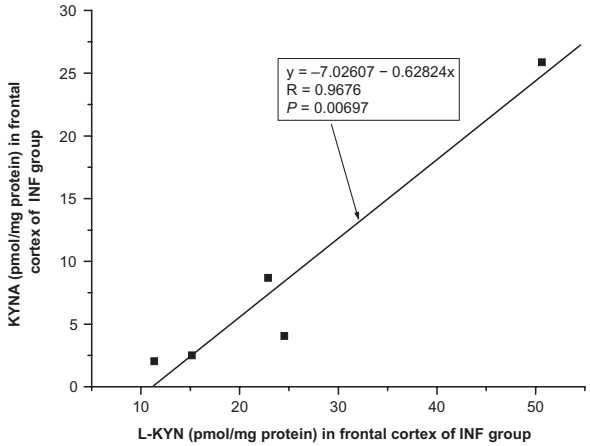

Figure 5. Relationship between kynurenic acid (KYNA) and L-kynurenine (L-KYN) in the frontal cortex of infarction of brain (INF, n = 5) group, one-way analysis of variance, F = 44.0554.

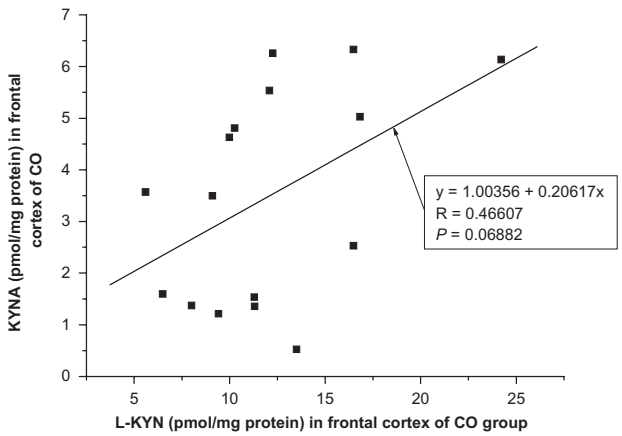

Figure 6. Relationship between kynurenic acid (KYNA) and L-kynurenine (L-KYN) concentration in the frontal cortex of control (CO, n = 16) group, one-way analysis of variance, F = 3.88506.

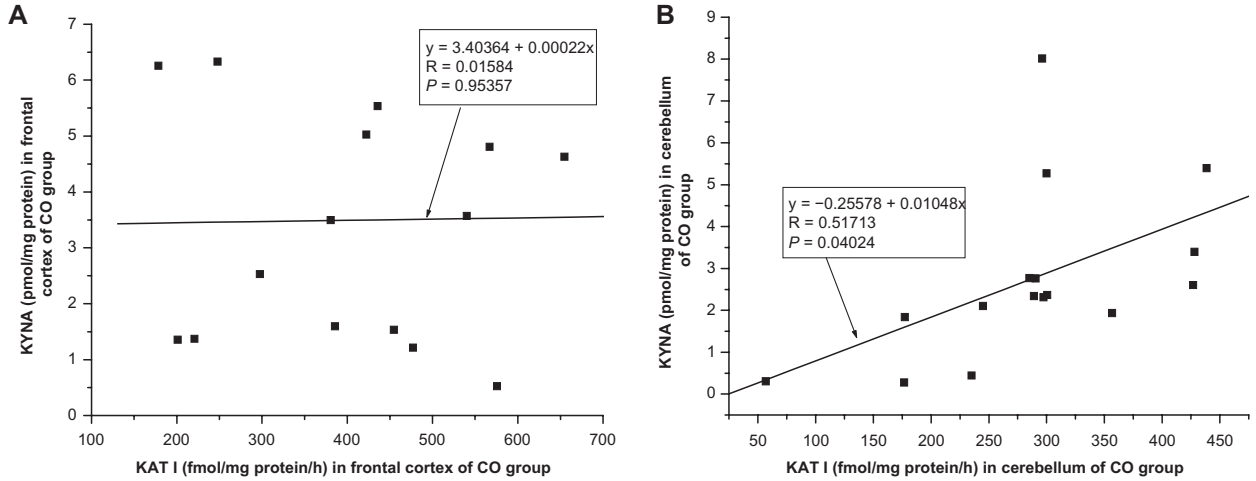

Figure 7 (**A** and **B**) Relationship between kynurenic acid (KYNA) level and kynurenine aminotransferase I (KAT I) activity in the frontal cortex, one-way analysis of variance, F = 0.00351 (**A**) and in the cerebellum, one-way analysis of variance, F = 5.11059 (**B**), of control (CO, n = 16) group.

and pathological subgroups HIV (R = –0.0506, F = 0.0103, *P* = 0.9242); 0PP (R = –0.1547, F = 0.3187, *P* = 0.5820); LY (R = –0.4748, F = 0.8732, *P* = 0.4190); INF (R = 0.3959, F = 0.55766, *P* = 0.5094) and GD (R = –0.2944, F = 0.2848, *P* = 0.6306), using one-way ANOVA.

Relationship between KAT I and KAT II in the frontal cortex and cerebellum

A significant correlation was found between KAT II and KAT I in the frontal cortex of INF (R = –0.8846, F = 10.7948, *P* = 0.0462, one-way ANOVA, Fig. 8A), a moderate correlation was found between KAT II and KAT I in the frontal cortex of GD (R = –0.7943, F = 5.1269, *P* = 0.1085) and of CO (R = 0.4219, F = 3.0313, *P* = 0.1036), and no significant correlation was seen in the frontal cortex of OPP (R = –0.3336, F = 1.6285, *P* = 0.2242); HIV (R = –0,1561, F = 0.0999, *P* = 0.7677) and LY (R = 0.0182, F = 0.9768, *P* = 0.9768), using one-way ANOVA. In the cerebellum, a correlation was stated between KAT I and KAT II of GD (R = –0.92328, F = 17.3318, *P* = 0.0252, one-way ANOVA, Fig. 8B) and a moderate correlation between KAT I and KAT II was seen in the cerebellum of HIV (R = 0.6799, F = 3.4400, *P* = 0.1372). No significant correlation was observed between KAT I and KAT II in the cerebellum of CO (R = 0.1963, F = 0.5614, *P* = 0.4661); OPP (R = 0.0452, F = 0.0266, *P* = 0.8729); LY (R = –0.1757, F = 0.0956, *P* = 0.7774) and INF

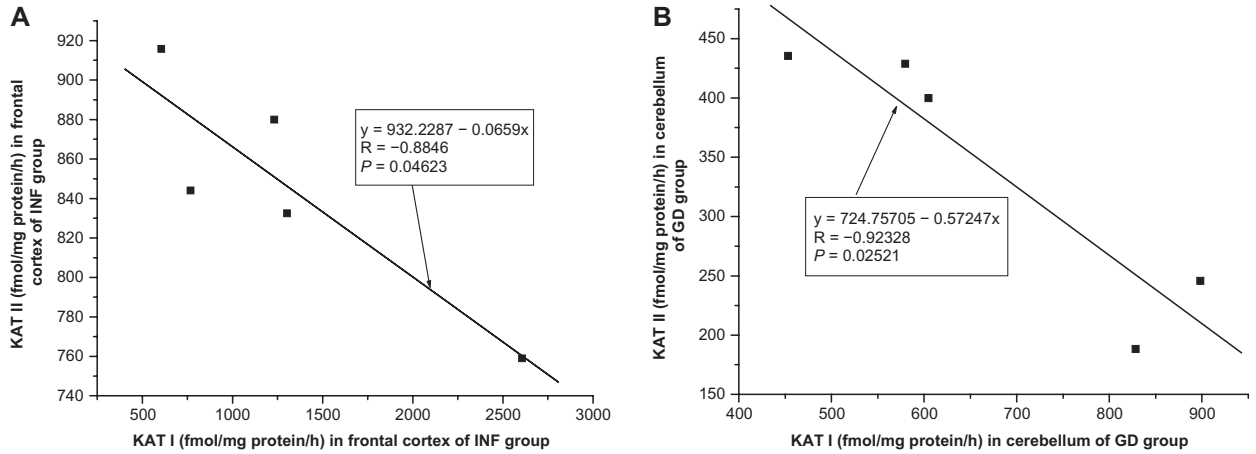

Figure 8 (**A**) Relationship between kynurenine aminotransferase II (KAT II) and kynurenine aminotransferase I (KAT I) in the frontal cortex of infarction of brain (INF, n = 5) group, one-way analysis of variance, F = 10.79478. (**B**) Relationship between kynurenine aminotransferase II (KAT II) and kynurenine aminotransferase I (KAT I) in the cerebellum of glial dystrophy (GD, n = 5) group, one-way analysis of variance, F = 17.3318.

(R = 0.3997, F = 0.5705, $P = 0.5049$), using one-way ANOVA.

Correlation between kynurenic acid and ratio KAT I/ KAT II in the frontal cortex and cerebellum

No significant correlation was found between kynurenic acid and ratio KAT I/KAT II in the frontal cortex of CO (R = –0.2130, F = 0.6652, $P = 0.4281$, Fig. 9A); OPP (R = –0.1077, F = 0.1530, $P = 0.7020$, Fig. 10A), HIV (R = –0.0808, F = 0.0263, $P = 0.8791$, Fig. 11A); LY (R = –0.4477, F = 0.7519, $P = 0.4496$, Fig. 12A); INF (R = –0.1336, F = 0.0545, $P = 0.8303$, Fig. 13A) and GD (R = –0.5121, F = 0.4939, $P = 0.5327$, Fig. 14A), using one-way ANOVA. No significant correlation was observed between kynurenic acid levels and ratio KAT I/ KAT II in the cerebellum of CO (R = 0.0455, F = 0.0290, $P = 0.8672$, Fig. 9B); OPP (R = –0.1847, F = 0.4593, $P = 0.5098$ Fig. 10B); HIV (R = 0.4055, F = 0.7872, $P = 0.4250$, Fig. 11B); INF (R = –0.6841, F = 2.6385, $P = 0.2028$, Fig. 13B) and GD (R = 0.2689, F = 0.66169, $P = 0.6617$, Fig. 14B), using one-way ANOVA, and only a moderate relationship between kynurenic acid and ratio KAT I/KAT II could be seen in the cerebellum of LY (R = 0.7770, F = 4.57207, $P = 0.12206$, Fig. 12B).

Correlation between L-kynurenine and ratio KAT I/KAT II in the frontal cortex and cerebellum

No relationship was found between L-kynurenine and ratio KAT I/KAT II in the frontal cortex of CO

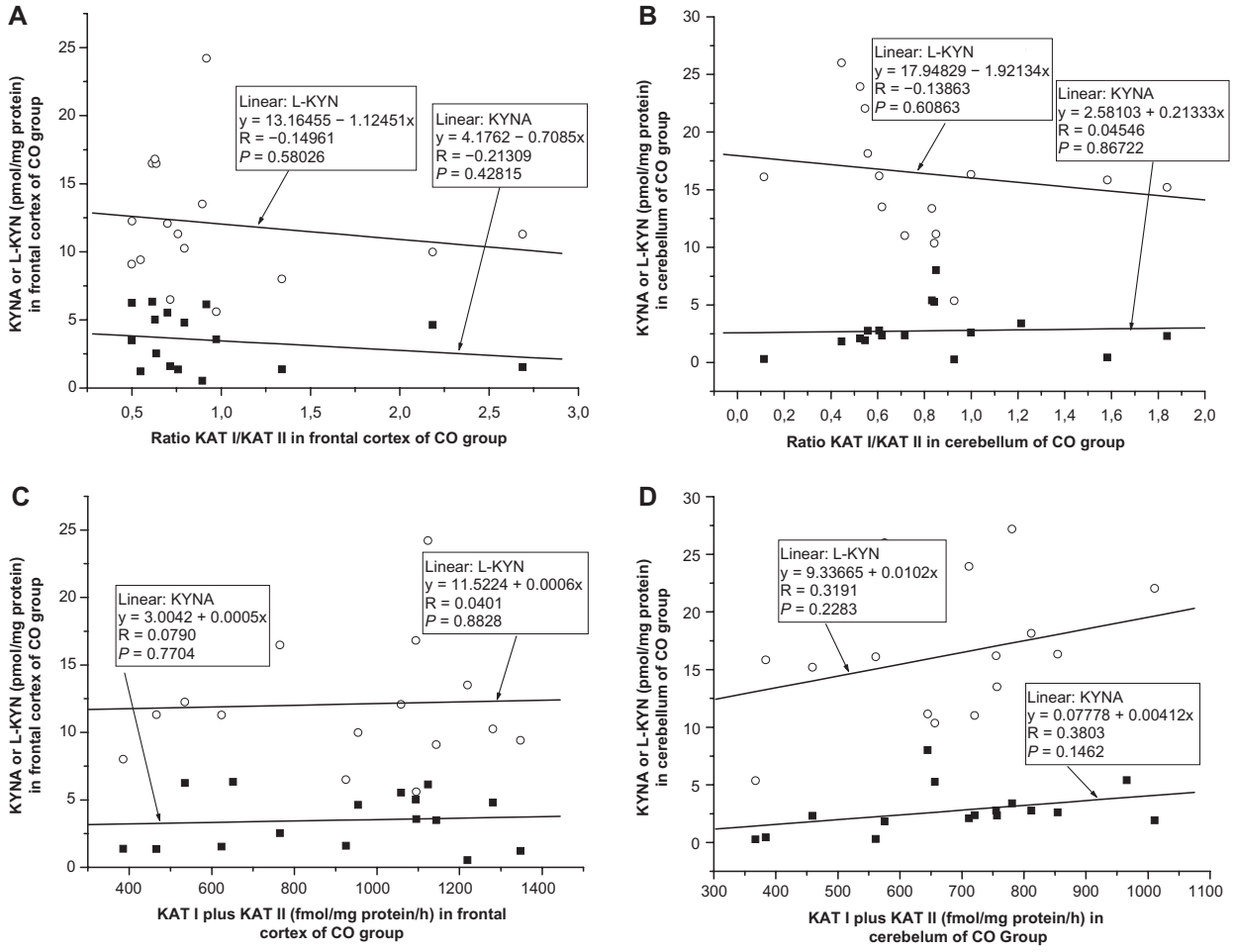

Figure 9 (**A** and **B**) Relationship between kynurenic acid (KYNA) or L-kynurenine (L-KYN) and ration of kynurenine aminotransferase I (KAT I)/ kynurenine aminotransferase I (KAT I) in the frontal cortex (**A**) and cerebellum (**B**) of control (CO, n = 16) group, using one-way analysis of variance. (**C** and **D**) Relationship between kynurenic acid (KYNA) or L-kynurenine (L-KYN) and combined activity of kynurenine aminotransferase I (KAT I) and kynurenine aminotransferase II (KAT II) in the frontal cortex (**C**) and cerebellum (**D**) of control (CO, n = 16) group, using one-way analysis of variance.

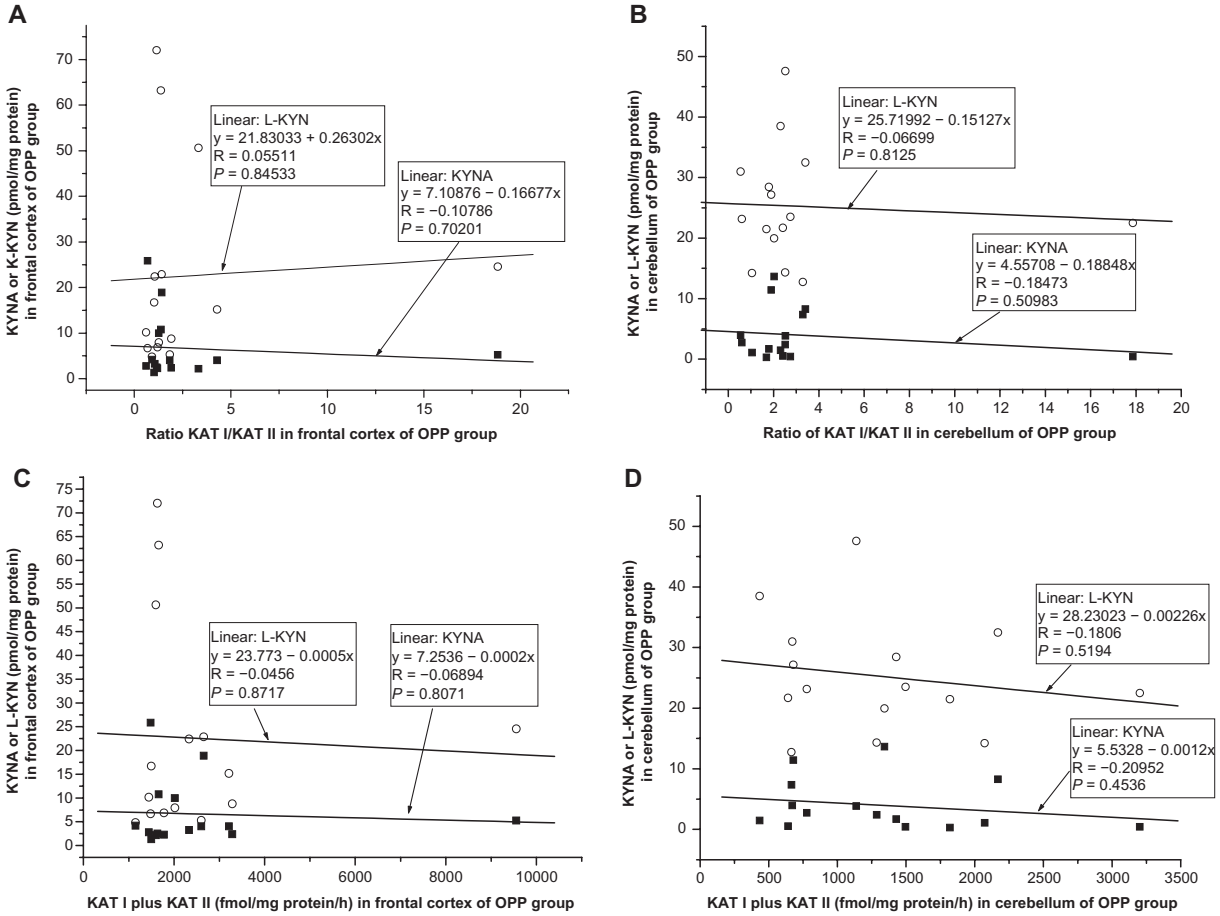

Figure 10 (**A** and **B**) Relationship between kynurenic acid (KYNA) or L-kynurenine (L-KYN) and ration of kynurenine aminotransferase I (KAT I)/kynurenine aminotransferase I (KAT I) in the frontal cortex (**A**) and cerebellum (**B**) of opportunistic infection (OPP, n = 15) group, using one-way analysis of variance. (**C** and **D**) Relationship between kynurenic acid (KYNA) or L-kynurenine (L-KYN) and combined activity of kynurenine aminotransferase I (KAT I) and kynurenine aminotransferase II (KAT II) in the frontal cortex (**C**) and in the cerebellum (**D**) of opportunistic infection (OPP, n = 15) group, using one-way analysis of variance.

(R = –0.1496, F = 0.3200, *P* = 0.5802, Fig. 9A); OPP (R = 0,0551, F = 0.0396, *P* = 0.8453, Fig. 10A), HIV (R = –0.3619, F = 0.6031, *P* = 0.4807, Fig. 11A); INF (R = –0.1973, F = 1215, *P* = 0.7504, Fig. 13A), and GD (R = –0.5956, F = 1.6492, *P* = 0.2893, Fig. 14A), using one-way ANOVA, but a correlation between L-kynurenine and ratio KAT I/KAT II was stated in the frontal cortex of LY (R = –0.8944, F = 12,0033, *P* = 0.0405, Fig. 12A). No significant correlation was observed between L-kynurenine levels and ratio KAT I/KAT II in the cerebellum of CO (R = –0.1386, F = 0.2743, *P* = 0.6086, Fig. 9B); OPP (R = –0.0669, F = 0.0586, *P* = 0.8125, Fig. 10B); LY (R = –0.2626, F = 0.2222, *P* = 0.6695, Fig. 12B); INF (R = –0.0617, F = 0.0115, *P* = 0.9215, Fig. 13B), and GD (R = –0.5564, F = 1.3455, *P* = 0.3299, Fig. 14B), using one-way ANOVA, and only a moderate relationship between L-kynurenine and ratio KAT I/KAT II was seen in the cerebellum of HIV (R = –0.6301, F = 2.6335, *P* = 0.1799, one-way ANOVA, Fig. 11B).

Relationship between kynurenic acid or L-kynurenine and combined KAT I and KAT II activities in the frontal cortex and cerebellum

Correlation between kynurenic acid or L-kynurenine and combined with KAT I and KAT II in the frontal cortex and cerebellum of all groups was analyzed using one-way ANOVA, and data are presented in Figure 9C and D; Figure 10C and D; Figure 11C and D; Figure 12C and D; Fig 13C and D; and Figure 14C and D.

High levels of KAT I/KAT II or high levels of combined KAT I and KAT II were associated with high kynurenic acid level and low L-kynurenine in

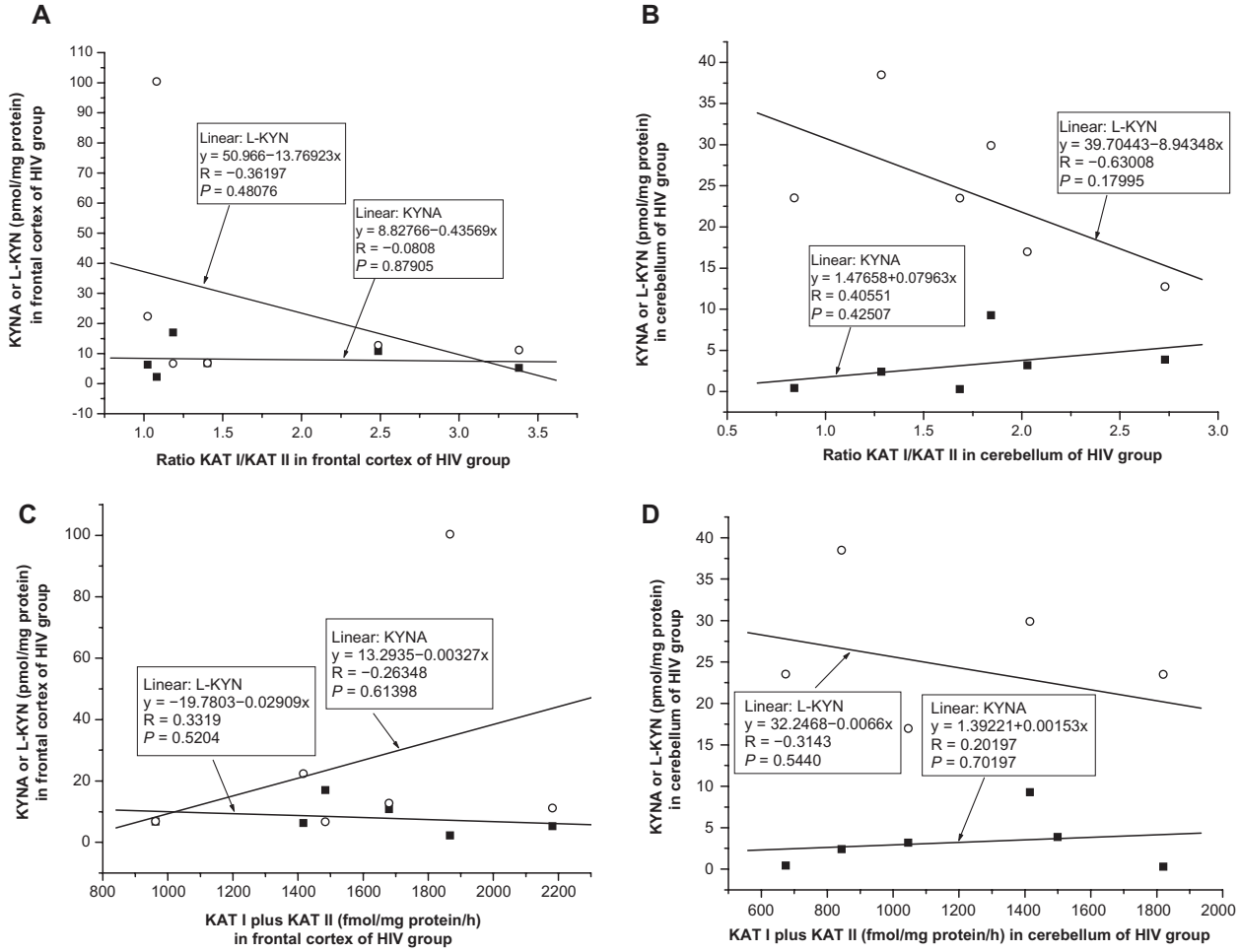

Figure 11 (**A** and **B**) Relationship between kynurenic acid (KYNA) or L-kynurenine (L-KYN) and ration of kynurenine aminotransferase I (KAT I)/kynurenine aminotransferase I (KAT I) in the frontal cortex (**A**) and cerebellum (**B**) of HIV-1 in brain tissue as found by anti-HIV immunocytochemistry (HIV, n = 6) group, using one-way analysis of variance. (**C** and **D**) Relationship between kynurenic acid (KYNA) or L-kynurenine (L-KYN) and combined kynurenine aminotransferase I (KAT I) and kynurenine aminotransferase II (KAT II) in the frontal cortex (**C**) cerebellum (**D**) of HIV-1 in brain tissue as found by anti-HIV immunocytochemistry (HIV, n = 6) group, using one-way analysis of variance.

the frontal cortex (Fig. 11A and C) and cerebellum (Fig. 11B and D) of HIV group, and also in the frontal cortex (Fig. 12C) and cerebellum (Fig. 12B and D) of LY group and in cerebellum of GD group (Fig. 14B). Whereas in the frontal cortex and cerebellum of OPP, INF and GD, high levels of KAT I/KAT II or high levels of combined KAT I and KAT II were associated with lower L-kynurenine levels and no changes of kynurenic acid or even moderate reduction, but this association could not be statistically proved.

One-way ANOVA between post mortem time or length of disease and kynurenic acid or L-kynurenine or KATs revealed no statistically significant correlation.

Discussion

We have previously reported increased kynurenic acid metabolism in the brain after HIV-1 infection.[11] Our results corroborate the findings of Heyes on increased kynurenic acid levels in the cerebrospinal fluid.[4,5] The present study extends the information on kynurenine metabolism in the brain considering the different types of pathology found after HIV-1 infection ie, HIV, OPP, INF, LY and GD.[20] The most frequent pathology was OPP (65%) followed by HIV (26%), LY (22%), INF (22%) and GD (22%). These observations are in line with previously published data.[19–21] An enhancement of kynurenic acid was present in the frontal cortex of all subgroups; however, the highest levels were measured in the LY, INF, and HIV. In contrast, in the cerebellum a significantly increased kynurenic acid content was seen only in the GD group, whereas in other pathological groups like LY it was moderately enhanced and in the OPP, INF and HIV it was comparable to controls. The fact that

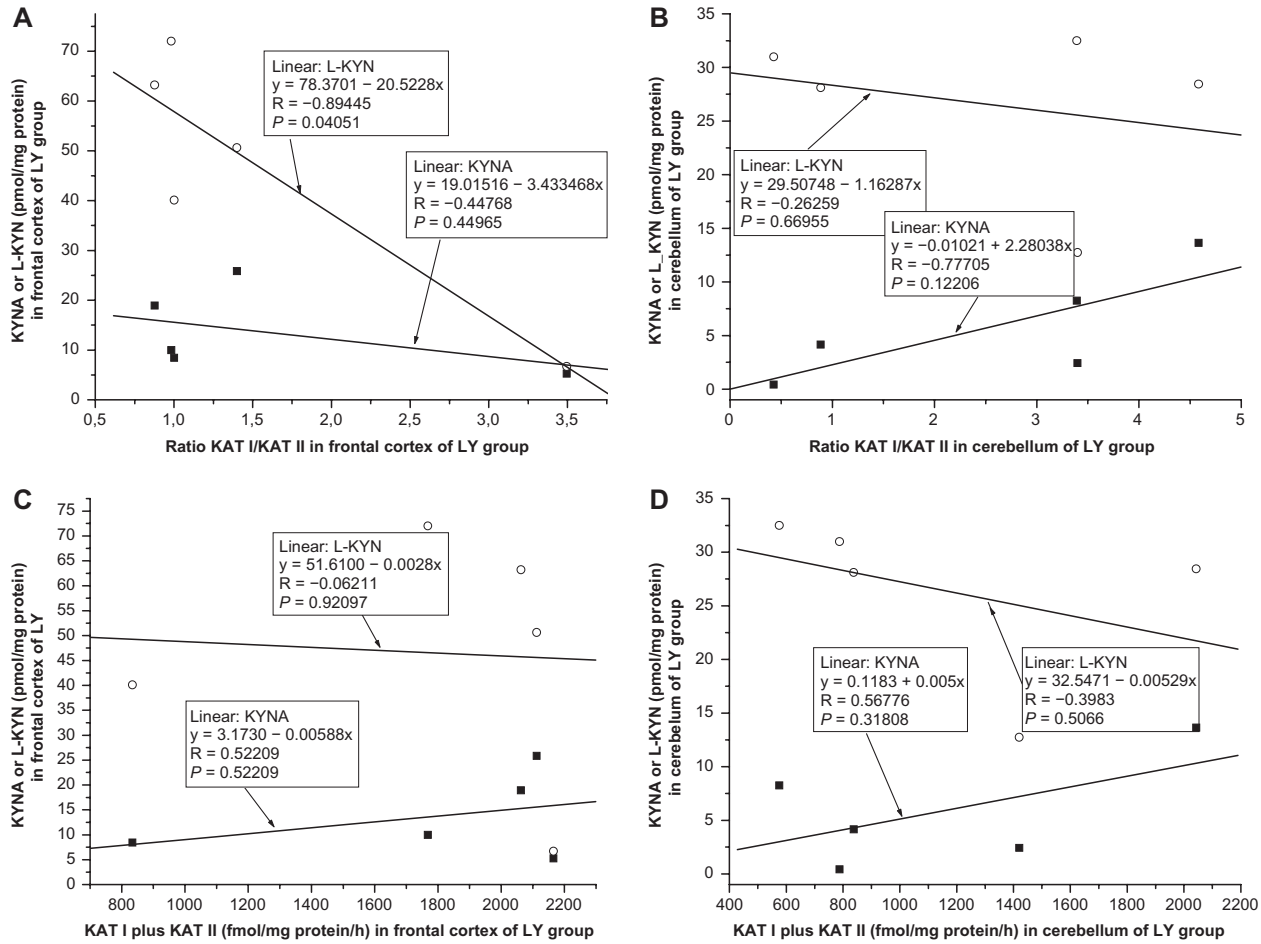

Figure 12 (**A** and **B**) Relationship between kynurenic acid (KYNA) or L-kynurenine (L-KYN) and ration of kynurenine aminotransferase I (KAT I)/kynurenine aminotransferase I (KAT I) in the frontal cortex (**A**) and cererbellum (**B**) of malignant lymphoma (LY, n = 5) group, using one-way analysis of variance. (**C** and **D**) Relationship between kynurenic acid (KYNA) or L-kynurenine (L-KYN) and combined activity of kynurenine aminotransferase I (KAT I) and kynurenine aminotransferase II (KAT II) in the frontal cortex (**C**) and cerebellum (**D**) of malignant lymphoma (LY, n = 5) group, using one-way analysis of variance.

different increases of kynurenic acid synthesis in the brain of HIV-1 infected patients may reflect several different reasonable conditions for its synthesis, including different pathologies. In addition, a weakening of the blood brain barrier in AIDS brain[20] might account for increased kynurenic acid permeation and of other metabolites, including L-kynurenine.[30] Furthermore, chronic immune stimulation of the brain with widespread microglia and astroglial activation may lead to excessive γ-interferon production[1,3,31] and subsequently induction of tryptophan metabolism, followed by an increase of L-kynurenine and enhancement of kynurenic acid levels in the brain.[23] Experimental data provides significant evidence that the increase of kynurenic acid easily takes place due to elevated L-kynurenine levels.[23,32] Indeed, our data revealed an increase of L-kynurenine in the frontal cortex of all pathologies, markedly of LY. However, the positive correlation between L-kynurenine and kynurenic acid was found in the frontal cortex of INF, LY, GD and CO, while negative correlation was stated in the frontal cortex of HIV and OPP. This might have an impact on the high ration of quinolinic acid/kynurenic acid shown in the CNS of HIV infected patients.[4,5] In the cerebellum of all pathologies, the content of L-kynurenine was enhanced moderately and significantly. The content of kynurenic acid, however, increased only in GD and LY and not in the HIV, OPP or INF. This finding allowed us to speculate the involvement of L-kynurenine in the synthesis of quinolinic acid rather than in the formation of kynurenic acid. Besides L-kynurenine involvement, other biochemical events have an impact on kynurenic acid formation. Enzymes responsible for kynurenic acid

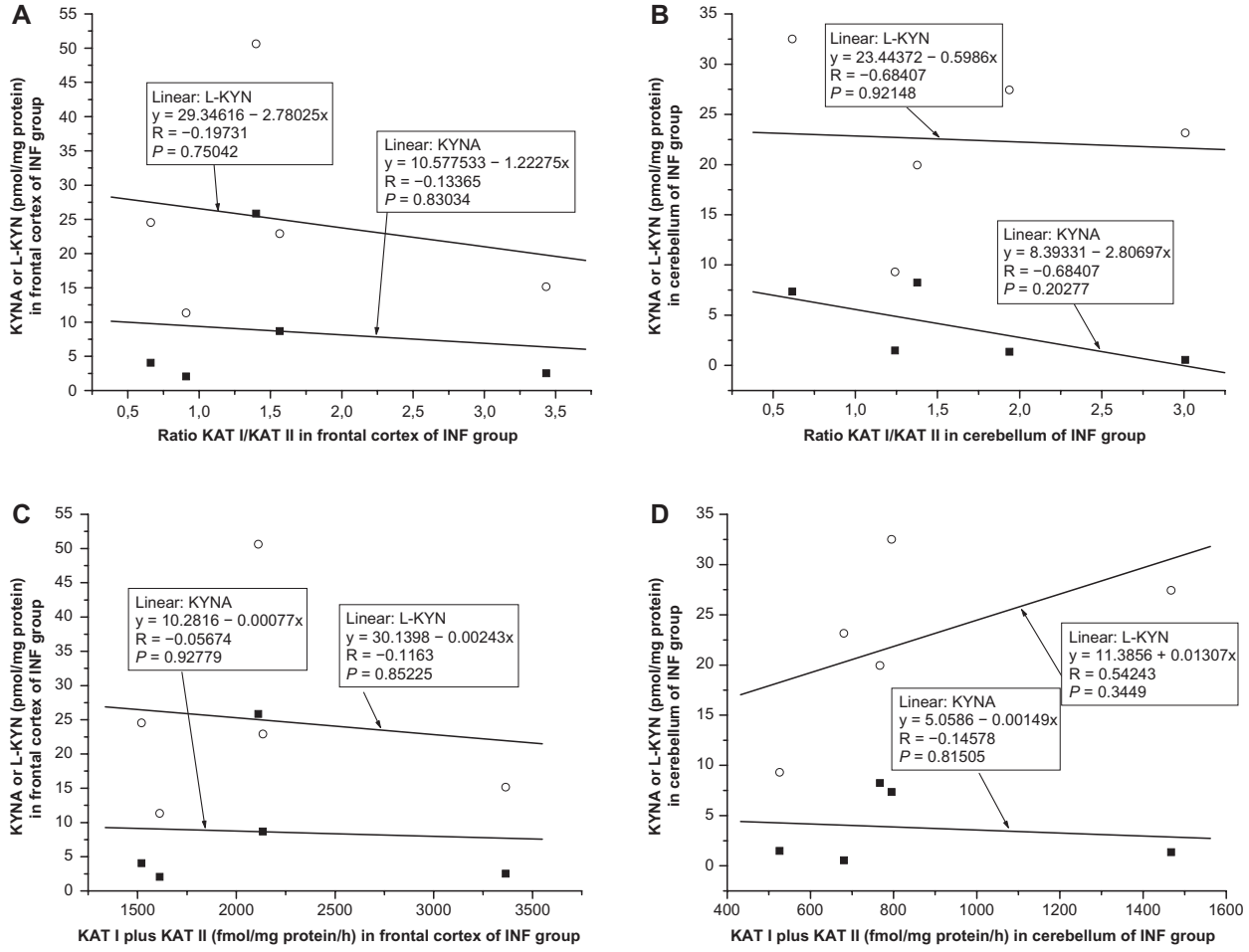

Figure 13 (**A** and **B**) Relationship between kynurenic acid (KYNA) or L-kynurenine (L-KYN) and ration of kynurenine aminotransferase I (KAT I)/ kynurenine aminotransferase I (KAT I) in the frontal cortex (**A**) and cerebellum (**B**) of infarction of brain (INF, n = 5) group, using one-way analysis of variance. (**C** and **D**) Relationship between kynurenic acid (KYNA) or L-kynurenine (L-KYN) and combined kynurenine aminotransferase I (KAT I) and kynurenine aminotransferase II (KAT I)) in the frontal cortex (**C**) and cerebellum (**D**) of infarction of brain (INF, n = 5) group, using one-way analysis of variance.

formation, KAT I and KAT II, are significantly and differently altered in the frontal cortex and cerebellum of all pathologies. Whereas, KAT I activity increased significantly in the frontal cortex and cerebellum of all pathologies, KAT II elevation was found only in the frontal cortex of INF and OPP. Interestingly, changes of kynurenic acid, L-kynurenine, KAT I and KAT II in the frontal cortex and cerebellum of INF group correlated well with published data of INF but not-HIV-1 subject.[14] Analysis on the relationship between kynurenic acid level and KAT I or KAT II activity changes in frontal cortex and cerebellum of all pathologies, however, revealed significant positive correlation between kynurenic acid level and KAT I activity only in the cerebellum of CO.

Surprisingly, analysis on the relationship between KAT II and KAT I activity revealed negative correlation between them in the frontal cortex of INF and GD, and in the cerebellum of GD, in comparison to positive correlation between KAT II and KAT I in the frontal cortex and cerebellum of CO group. A positive correlation between KAT II and KAT I activity was observed in the cerebellum of HIV. In order to find more understanding in the difference between pathological subgroups, the relationship between the ration of KA I/KAT II or combined activity of KAT I and KAT II, and kynurenic acid or L-kynurenine was analyzed. Interestingly, in the frontal cortex and cerebellum of the HIV and LY group, we found association between high enzyme activity and increased kynurenic acid and lower L-kynurenine. Whereas, in the frontal cortex and cerebellum of GD group, the high ratios of KAT I/KAT II were associated with low L-kynurenine and low kynurenic acid. Furthermore, in the frontal cortex and cerebellum of CO and OPP group, both L-kynurenine and kynurenic acid were

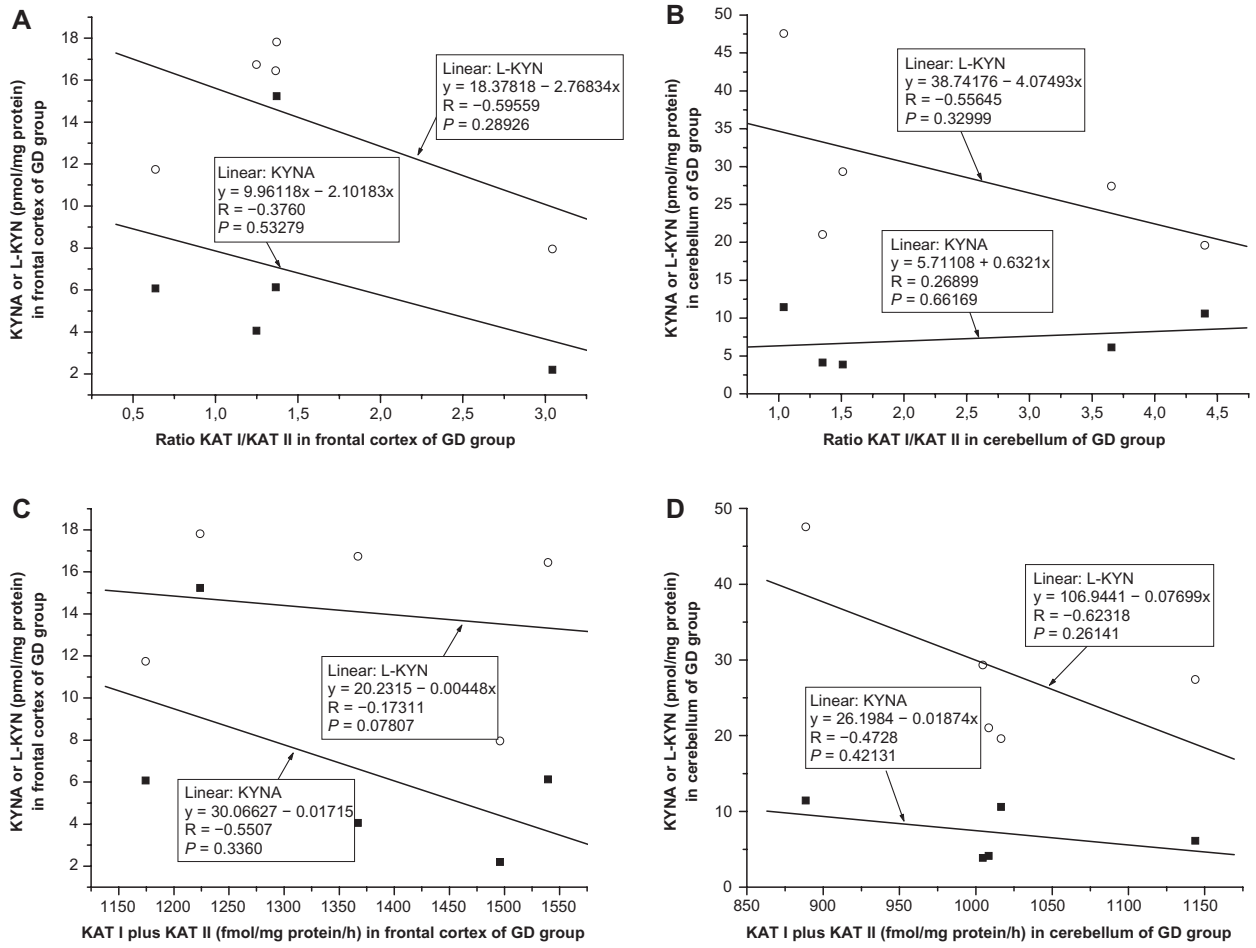

Figure 14 (**A** and **B**) Relationship between kynurenic acid (KYNA) or L-kynurenine (L-KYN) and ration of kynurenine aminotransferase I (KAT I)/ kynurenine aminotransferase I (KAT I) in the frontal cortex (**A**) and cerebellum (**B**) of glial dystrophy (GD, n = 5) group, using one-way analysis of variance. (**C** and **D**) Relationship between kynurenic acid (KYNA) or L-kynurenine (L-KYN) and combined activity of kynurenine aminotransferase I (KAT I) and kynurenine aminotransferase II (KAT II) in the frontal cortex (**C**) and cerebellum (**D**) of glial dystrophy (GD, n = 5) group, using one-way analysis of variance.

not or only moderately associated with high ratio of KAT I/KAT II. This data is interesting and might have a functional meaning, however further study with a higher number of pathological cases are necessary to prove this observation.

The highest increases of KAT I (460% of CO) and KAT II (160% of CO) found in the frontal cortex of OPP were accompanied by moderate increase of kynurenic acid levels. It is not known if widespread reactive astrocytosis in the human HIV-1 brain and elevated KAT I activity are common occurrences. It has been noted previously that in the putamen and caudate nucleus of Alzheimer diseased brains, KAT I activity is increased.[14] Interestingly, these regions are both characterized by astrocytosis.[33,49] It is therefore questionable if common insight exists between the noticed increases in KAT I activity and astrocytosis in the frontal cortex of HIV-1 patients.[20,34] Marked elevation of KAT I activity in the brain indicates activation and/or proliferations of astrocytes.[20] On the other hand, if we believe that KAT II is the enzyme which affects physiological functions, its increase in the frontal cortex, particularly of OPP and INF, might have adaptive physiological functions after HIV-1 infection, on contrary to lacking increase of KAT II in the cerebellum.

Bronchopneumonia, lobar pneumonia, oedema of the lung, or tuberculosis occurs frequently after HIV-1 infection, an observation in line with previously published data.[35,36] Our data for the first time demonstrates a marked increase of KAT I in the frontal cortex and cerebellum of non-HIV infected subjects (control cases) with pathology of bronchopneumonia. These observations allow us to postulate that pneumonia and/or bronchopneumonia might have a significant impact on kynurenic

acid metabolism in the CNS. No increase of KAT II activity has been seen in the brains of these patients with bronchopneumonia and, in good correlation, among pathological HIV-1 subgroups the increase of KAT II was only moderate. Furthermore, in the model of encephalomyocarditis (EMCV) by piglets, after infection with Picornea virus, we found breeding difficulty, circulation insufficiency, depression, and a marked increase of kynurenic acid levels in the blood.[37] It has also been found that the infection with Picornea virus is accompanied by increased kynurenic acid, but not by dramatic changes of enzyme activity in the brain, at least in the acute phase. Furthermore, the disease was characterized by high lethality of piglets.[37,38] An increased kynurenic acid level in the brain or serum seems to be a confounding factor related to lethality by HIV-1 patients, too. A high mortality has been reported in patients infected with HIV-1 virus[21] and this may be related to increased kynurenic acid, which plays a pivotal role with respect to cardio-respiratory function. In our previous study, we have shown that an increase in kynurenic acid lowered oxygen consumption of the heart mitochondria and ATP synthesis.[39] In addition, another study showed that a deficit in oxygen, due to asphyxia, caused a marked increase of kynurenic acid in the brain.[40] Also of interest is the finding that the longer the period of oxygen deficit, the higher the lethality and the higher the observed peak of kynurenic acid in the brain.[40]

A growing amount of data indicates that increased kynurenic acid formation in the brain is involved in the development of neuropsychiatric diseases, such as Alzheimer's, Downs Syndrome, and Schizophrenia and dementia.[14,41–43] A high probability for development of dementia due to CNS lymphoma in the CNS has been described.[44] The occurrence of dementia in HIV-1 patients with INF[44] has been reported, too, and elderly people with silent brain infarcts have an increased risk of dementia.[45] In this line, increased kynurenic acid has been found in the CSF of elderly patients.[15] Additionally, in the other subgroup of pathology, like OPP or HIV, the development of dementia and/or cognitive impairment have been well documented.[19–21,46] Thus, a marked and permanent increase of kynurenic acid in the frontal cortex of LY might have contributed to the development of dementia. In this regard, the action of antidementia agent Cerebrolysin[47,48] involves lowering of kynurenic acid formation in the human brain,[15] although its mechanism of action needs to be still clarified. It is important to mention that also quinolinic acid plays a crucial role in the development of dementia after HIV-1 infection.[8] Unfortunately quinolinic acid was not investigated in this study due to limited capacity of HPLC method.

In summary, the present data demonstrates differences in kynurenic acid metabolism of the frontal cortex and cerebellum among different pathological groups of HIV-1 patients. OPP was the most frequent pathology with relatively selective alterations of kynurenine metabolites. The neurochemical changes seen in the brains of different pathological groups after HIV-1 infection correlates in part with the neurochemical changes described by different neurological conditions. We suggest that modulation of kynurenic acid synthesis by lowering kynurenic acid synthesis supports the cardio-respiratory system and acts, at least in part, as an antidementia agent. However, it is still questionable if conditions related to respiratory deficits, dementia and lethality share dose dependent involvement when it comes to the increased kynurenic acid metabolism observed.

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Author Contributions

Conceived and designed the experiments: H.B. Analysed the data: H.B and J.A.H. Wrote the first draft of the manuscript: H.B. Contributed to the writing of the manuscript: J.A.H and B.K. Agree with manuscript results and conclusions: H.B., J.B.K., B.K. Jointly developed the structure and arguments for the paper: H.B., J.B.K., B.K. Made critical revisions and approved final version: H.B., J.B.K., B.K. All authors reviewed and approved of the final manuscript.

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Competing Interests

Author(s) disclose no potential conflicts of interest.

Disclosures and Ethics

As a requirement of publication author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest.

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