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Annals of Psychiatry and Mental Health

Short Communication

Anthranilic Acid: A Potential Biomarker and Treatment Target for Schizophrenia

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Submitted: 08 January 2016

Accepted: 28 January 2016

Published: 30 January 2016

ISSN: 2374-0124

OPEN ACCESS

Keywords
- Anthranilic acid
- Schizophrenia
- Kynurenines
- Tryptophan
- Kynurenic Acid

Abstract

Dysregulation of Trp - Kyn pathway is a recent hypothesis of mechanisms of schizophrenia. In particular, over-production of kynurenic acid (KYNA), one of the three immediate downstream metabolites of kynurenine (Kyn) along tryptophan (Trp) – Kyn pathway, has been considered as a new target for therapeutic intervention in schizophrenia. Up-regulation of KYNA formation was suggested to occur at the expense of down-regulation of 3-hydroxyKyn (3-HK), the other immediate downstream metabolite of Kyn. We were interested to assess the fate of the third immediate downstream Kyn metabolite, anthranilic acid (AA). Serum AA concentrations were evaluated by HPLC-mass spectrometry method in patients with schizophrenia and control subjects. We found 5-fold increase of AA and 3-fold decrease of 3-HK concentrations in serum of schizophrenia patients. Impact of AA elevation in schizophrenia might be mediated by mitochondrial enzymes down regulation described in schizophrenia, and upregulation (found in anterior cingulate brains of schizophrenia) of formation of 3-hydroxy AA, a potent generator of free radicals and glutamatergic agonists. Present data warrant further studies of AA as biological marker of, at least, a subgroup of schizophrenia patients and as a potential new target for therapeutic intervention.

ABBREVIATIONS

Trp : Tryptophan; Kyn : Kynurenine; KYNA : Kynurenic acid; AA : Anthranilic Acid; 3-HK : 3-HydroxyKynurenine; IDO - Indoleamine 2,3-dioxygenase; TDO : Tryptophan 2,3-dioxygenase; KMO - Kynurenine 3-Monoxygenase; KAT - Kynurenine Amino Transferase ; Kynase : Kynureninase; NAD+ : Nicotinamide Adenine Dinucleotide

INTRODUCTION

Schizophrenia is a severe and debilitating psychiatric disorder with 1% lifetime prevalence, relatively independent of geographic, cultural, and socioeconomic variables. The pathogenetic mechanisms of schizophrenia, which have not been well defined, have the potential to hold a key to treatment, necessitating further investigation as a critical step in understanding this devastating mental illness. Currently, dysregulation of kynurenine (Kyn) pathway of tryptophan (Trp) metabolism is considered as a potential pathogenetic mechanism of schizophrenia [1]. Trp – Kyn pathway consists of Trp conversion into Kyn, a common precursor of 3-hydroxykynurenine (3-HK), kynurenic (KYNA) and anthranilic (AA) acids. 3-HK is further catalyzed into 3-hydroxyanthranilic acid (3-HAA), and, eventually, NAD^+ (Figure 1) [2].

Postmortem studies revealed inhibition (unrelated to use of antipsychotic medication) of mRNA and activity of kynurenine-3-monooxygenase (*KMO*) enzyme catalyzing Kyn conversion into 3-HK in the prefrontal cortex (brain area implicated in schizophrenia) of schizophrenia patients [3]. *KMO* inhibition elevates content of KYNA, an endogenous antagonist to N-methyl-D-aspartate (NMDA) and a7-nicotinic acetylcholine (a7-ach) receptors, in brains and CSF of schizophrenia patients by increasing availability of Kyn as a substrate for kynurenine aminotransferase (*KAT*), catalyzing formation of KYNA from Kyn (Figure 1) [4,5]. KYNA triggers relapses in remitted schizophrenia patients and induces schizophrenia-like symptoms in animals models of schizophrenia, supporting "KYNA hypothesis of schizophrenia" (Figure 1A) [6,7].

However, concurrently with up regulation of KYNA production, inhibition of *KMO* activates Kyn conversion into AA, catalyzed by AA-kynureninase (*Kynase*) (Figure 1). Since *Kynase* (and *KAT*) are substrate-unsaturated enzymes, they are able to process additional amount of Kyn created by *KMO* inhibition.

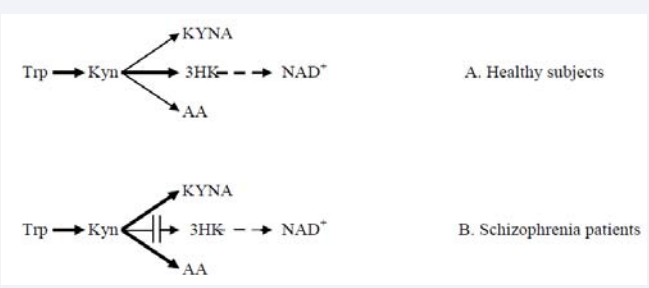

Figure 1 Proposed down-stream metabolism of kynurenine in schizophrenia
Abbreviations: Trp: Tryptophan; Kyn : Kynurenine; KYNA : Kynurenic acid; AA : Anthranilic Acid; 3-HK : 3-HydroxyKynurenine; NAD+ : Nicotinamide Adenine Dinucleotide

Thus, *KMO* inhibition induced by diet low on a *KMO* co-factor, riboflavin, increases urine excretion of both AA and KYNA in baboons and rats [8,9]. Similarly, elevation of KYNA and AA was described in the brain, liver, and plasma of *Kmo* knock-out ($Kmo^{-/-}$) mice [10].Therefore, data found in existing literature (i.e., *KMO* inhibition in schizophrenia patients and elevation of AA in animals with deficient *KMO*) suggest AA (in addition to KYNA) elevations in schizophrenia. However, the KYNA hypothesis did not consider possible role of AA elevation in mechanisms of schizophrenia. Consequently, the potential significance of AA elevation for pathophysiology of schizophrenia has not to date attracted the attention of researchers. We are aware of only one existing report of plasma AA elevation in a rat model of schizophrenia (social isolation rearing) [11], and one study that did not find any changes of AA plasma concentrations in patients with schizophrenia. However, method (electrochemical detector) used in this study was not specific and sensitive enough for AA determination [12,13].

Here we present preliminary results of our study of serum concentrations of downstream Kyn metabolites in schizophrenia patients.

MATERIALS AND METHODS

Patients

Overnight fasting blood samples were collected from patients (three men and three women, age range from 38 to 56 years) with schizophrenia, diagnosed according to DSM-V criteria [7]. All patients were taking anti-psychotic medication: Abilify (three patients), Haloperidol (one patient), Haloperidol decanoate injections (one patient) and Saphris (one patient).

Healthy Subjects (Controls)

There were 12 subjects (6 females and 6 males, age range from 32 to 64 years). Study was approved by Tufts Medical Center IRB.

Assessment of kynurenine metabolites

Serum samples were stored at −50˚C until analysis. AA, Trp, Kyn, KYNA and 3-HK concentrations were analyzed by modified HPLC–mass spectrometry (MS) method [13,14] used our previously published studies [15,16].

Statistical Analysis

Results are presented as mean ± standard error (Trp and Kyn in μM and AA, KYNA and 3-HK in nM). Statistical significance was assessed by Mann-Whitney test, two- tailed.

RESULTS AND DISCUSSION

Serum concentrations of Kyn and its metabolites

Assessment of peripheral AA is an appropriate approach in clinical studies considering that AA, in difference with KYNA, easily penetrates blood-brain barrier (via passive diffusion), and, therefore, peripherally produced AA might contribute significantly to it brain pools and act on targets in brain [17].

AA concentrations were increased (approximately five-fold) in schizophrenia patients in comparison with control subjects. AA increase was robust: all controls had AA serum concentrations lower than schizophrenia patients (Table 1).

3-HK concentrations were approximately three-fold lower in schizophrenia patients than in controls.

There was no statistically significant difference between Trp, Kyn and KYNA concentrations in serum of schizophrenia patients and controls.

Table 1: Down-stream kynurenine metabolites in serum of schizophrenia patients.

	Schizophrenia* (n=6)	Control* (n=12)	P(Mann-Whitney test, two-tailed)
Tryptophan (μM)	77.88±7.53	68.90± 2.49	ns
Kynurenine (μM)	2.06±0.27	1.76± 0.09	ns
Kyn x 100 : Trp	2.64±0.21	2.56±0.35	ns
Anthranilic acid (nM)	111.43±7.56	21.65±5.99	0.0001
AA : Kyn	60.89±11.98	12.22±3.22	0.0001
3-HK (nM)	6.25±0.05	19.55± 3.14	0.03
3-HK : Kyn	3.14±1.34	11.58±1.3	0.0003
KYNA (nM)	36.43±3.99	35.78± 3.59	ns
KYNA : Kyn	18.33±1.47	19.76±1.69	Ns

*mean± standard error
Abbreviations: Kyn : Kynurenine; Trp : Tryptophan; AA : Anthranilic Acid; 3-HK : 3-HydroxyKynurenine; KYNA : Kynurenic acid.

End product: substrate ratios of Trp – Kyn metabolic pathway

In clinical studies, evaluation of end product: substrate ratios are used for assessment of activities of enzymes, catalyzing corresponding metabolic reactions [18].

AA: Kyn ratio (marker of *Kynase* activity) was higher (1.6 fold) while 3-HK : Kyn ratio (marker of *KMO* activity) was lower (3.7 fold) in patients than in controls.

KYNA: KYN ratio (marker of *KAT* activity) did not differ between patients and controls (Table 1).

Kyn : Trp ratio, a clinical index of IDO and TDO activity, did not differ between patients and controls. Notably, elevated serum AA concentrations might limit utilization of Kyn: Trp ratio for assessment of IDO/TDO activity considering that AA is not only metabolite of Trp but a substrate of Trp formation by bacteria, e.g., by intestinal microbiome [19].

The main finding of the present communication is a robust and dramatic increase of AA concentrations in serum of a small sample of patients with schizophrenia. To the best of our knowledge, this is the first observation of increased AA concentrations in schizophrenia patients. Our results are in agreement with previously reported AA elevation in rat plasma in social isolation rearing model of schizophrenia [11]. It is unlikely that AA elevation depends on the use of anti-psychotic medication, considering that chronic administration of anti-psychotic drug risperidone did not affect Trp – Kyn metabolism in rat brain [3]. Elevation of AA concentrations could be caused by up-regulation of enzyme (*Kynase*), catalyzing conversion of Kyn into AA. Our finding of increased AA: Kyn ratio suggests up-regulation of *Kynase* activity. However, direct assessment of brain *Kynase* activity did not reveal its up-regulation in schizophrenia patients [3]. In the same vein, diet deficient of vitamin B6, a cofactor of *Kynase*, did not decrease urine AA excretion in baboons [8]. The other cause of AA elevation might be an increased formation of Kyn, a substrate for AA synthesis, from Trp, catalyzed by IDO/TDO, and/or on decreased conversion of Kyn into KYNA or 3-HK. We did not find increased Kyn: Trp (index of IDO/TDO activity) or decreased KYNA: Kyn ratio (index of *KAT* activity) in schizophrenia patients in agreement with observation of unchanged *KAT* activity in Broadmann areas of schizophrenia patients [3]. We did find decreased 3-HK : Kyn ratio (index of *KMO* activity) and drastic decrease of 3-HK serum concentrations in schizophrenia patients in agreement with previously reported reduction in *KMO* gene expression and *KMO* enzyme activity and 3-HK decrease in Broadmann areas of schizophrenia patients [3,4]. Administration of diet deficient of riboflavin, a *KMO* cofactor, increased AA (12-fold) and decreased 3-HK (10-fold) urine excretion in baboons suggesting that increased availability of substrate, Kyn, due to inhibition of *KMO*, up-regulates AA formation [8]. Effect of *KMO* inhibition on AA formation might be further supported by observation of increased AA excretion in riboflavin deficient rats [15] and AA elevation in brain, liver and plasma KMO(-/-) mice [10].

Therefore, the most likely cause of AA increase in schizophrenia is a shift of downstream Kyn metabolism from formation of 3-HK towards production of AA due to inhibition of *KMO*, an enzyme catalyzing Kyn conversion into 3-HK (Figure 1B).

Potential AA contribution to the pathogenesis of schizophrenia might be different from that of KYNA, an endogenous NMDA and a-7-ach antagonist [2]. Since no receptors have been yet ascribed to AA in mammalian systems, the effects of AA might be mediated by non-receptors mechanisms. Thus, AA-induced inhibition of rat brain complexes I-III of the respiratory chain [19] and reduction of state III oxygen consumption rate and suppression of mitochondrial respiratory control index [20,21] suggest that AA, synthesized in the outer membrane of mitochondria, augments oxidative stress and mitochondrial enzymes deficiency described in schizophrenia [22].

Another potential impact of elevated AA on pathophysiology of schizophrenia might be related to ability of peripherally formed AA to penetrate blood-brain barrier (BBB) (similarly to Trp, Kyn and 3-HK but in difference to KYNA and 3-HAA) [17].

Notably, inhibition of *KMO* in schizophrenia is expected to decrease the availability of 3-HK as a substrate for 3-HAA formation (Figure 1B). On the contrary, elevated 3-HAA content was found in postmortem anterior cingulate brain of schizophrenia (but not bipolar disorder) patients [23], suggesting 3-HAA formation from different than 3-HK substrate. Indeed, in rat brains *AA* was 10-fold superior to 3-HK as a bioprecursor of 3-HAA [24], and should, therefore, be much better suited to sustain cerebral 3-HAA levels when 3-HK formation is compromised (as in case of schizophrenia, in riboflavin-deficient diet, and in $Kmo^{-/-}$ mice.

3-HAA, a potent free radical generator and highly reactive compound in its own right, readily auto-oxidizes to form various intermediate oxidation products including cinnabarinic acid, an agonist of NMDA receptors [25]. Therefore, prevention of central formation of 3-HAA by inhibition of peripheral AA production might be useful in treatment of schizophrenia.

CONCLUSION

Preliminary report from our study reveals a drastic and robust elevation of AA serum concentrations in schizophrenia patients. Dysregulation of Trp - Kyn pathway is a recent hypothesis of mechanisms of schizophrenia. However, possible involvement of AA in mechanisms of schizophrenia has not been considered so far. Present data warrant further studies of AA as biological marker of, at least, a subgroup of schizophrenia patients and as a new target for therapeutic intervention in schizophrenia.

ACKNOWLEDGEMENTS

GF Oxenkrug is a recipient of NIMH104810 grant

CONFLICT OF INTEREST

P. Summergrad is a non-promotional speaker for CME outfitters, Inc. Other authors have nothing to declare.

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Cite this article

Oxenkrug G, van der Hart M, Roeser J, Summergrad P (2016) Anthranilic Acid: A Potential Biomarker and Treatment Target for Schizophrenia. Ann Psychiatry Ment Health 4(2): 1059.