Tryptophan Metabolism in Post-Withdrawal Alcohol-Dependent Patients

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(Received 2 December 2013; first review notified 29 January 2014; in revised form 13 February 2014; accepted 18 February 2014)

Abstract — Aims: The aim of the study was to investigate the parameters of tryptophan and phenylalanine metabolism and their associations to immune system activation and to behavioural symptoms during medium-term withdrawal (4–12 weeks of abstinence) in alcohol-dependent patients. Methods: Biochemical assays and clinical assessments at the beginning of treatment (fourth week of alcohol abstinence in average) and prior to the discharge after 8 weeks of treatment. Results: Kynurenine to tryptophan ratio (Kyn/Trp) slightly correlated with neopterin levels in early post-withdrawal period (Week 4 of abstinence) but this association disappeared after 12 weeks of abstinence. Phenylalanine and tyrosine concentrations as well as phenylalanine to tyrosine ratio (Ph/Trp) decreased between Weeks 4 and 12 of abstinence. Kynurenine and Kyn/Trp increased significantly at 12th week of abstinence when compared with the beginning of the study (Week 4 of abstinence). At Week 12, Kyn/Trp significantly correlated with such behavioural symptoms as fatigue, irritability and sleep disturbances. Conclusions: Tryptophan breakdown in early stages may be influenced by the increased activity of indoleamine 2,3-dioxygenase but the increase of Kyn/Trp between Weeks 4 and 12 of abstinence seems to be independent of immune changes and correlates with behavioural symptoms in later stages of the post-withdrawal course. A possible role of kynurenine metabolites in mediation of the increased stress sensitivity in post-withdrawal alcohol-dependent patients is discussed.

INTRODUCTION

Alcohol-dependent patients in the early post-withdrawal period suffer from a variety of symptoms including anxiety, craving, sleep disorder, irritability, mild depressive symptoms and high stress sensitization (Heilig et al., 2010). These symptoms persist for weeks to months after the acute alcohol withdrawal and substantially raise the risk of alcohol relapse. Although the relation of these clinical phenomena to alcohol addiction is apparent, their mechanisms are not fully understood. Most previous animal and human research has been concentrated on the acute alcohol withdrawal (1–14 days after the last alcohol intake). Acute withdrawal is characterized by severe glutamate-GABA imbalance (Tsai et al., 1995) as well as by disturbances of the immune system and various behavioural symptoms. Recent research focuses on the medium-term (several weeks) and long-term (several months) post-withdrawal period in order to elucidate the mechanisms of the recovery as well as to address the variety of behavioural symptoms abstinent alcohol patients demonstrate during the medium- and long-term rehabilitation.

Involvement of the immune system in various alcohol-associated disorders has attracted considerable research interest throughout the last decade. Recent findings have shown that chronic excessive alcohol consumption is associated with disturbances in a number of immune pathways which seem to play a substantial role in alcohol-associated behavioural symptoms (Redwine et al., 2003), neurodegeneration and neurotransmitter imbalance (Kelley and Dantzer, 2011). In this context, the understanding of tryptophan metabolism has been of particular interest because of its precursor role for serotonin synthesis. Under normal conditions, tryptophan degradation occurs through the combined activity of the hepatic tryptophan 2,3-dioxygenase (TDO) and the indoleamine 2,3-dioxygenase (IDO). In the absence of immune activation, the IDO activity constitutes ~5–15% of that of hepatic TDO (Badawy, 2013).

The activity of TDO seems to be sensitive to cortisol (Oxenkrug, 2010; Badawy, 2013) while IDO activation occurs mostly through cytokines (Schröcksnadel et al., 2006).

Previous reports have shown disturbances in tryptophan-kynurenine metabolism (Branchez and Lieber, 1982; Branchez et al., 1984; Friedman et al., 1988) and alcohol-induced inhibition of the hepatic TDO (Branchez, 2002). In contrast, the role of the IDO in alcoholism remains widely unknown. Immune-mediated activation of the IDO and the resulting changes in tryptophan and kynurenine metabolism have been suggested to contribute to the various neurologic and psychiatric diseases including depression and anxiety and are associated with depressive symptoms in chronically ill patients and the elderly (Schröcksnadel et al., 2006; Capuron and Miller, 2011; Haroon et al., 2012). Increased conversion of tryptophan to kynurenine at the expense of serotonin synthesis has been suggested as possible depressogenic mechanism (Schröcksnadel et al., 2006; Haroon et al., 2012).

In addition to the influence of inflammation and immune activation on tryptophan metabolism, an impact on phenylalanine metabolism has been described recently (Neurauter et al., 2008; Haroon et al., 2012). Altered phenylalanine metabolism has been reported to correlate with neuropsychiatric symptoms in elderly individuals (Capuron et al., 2011) as well as in patients with hepatitis C virus infection under therapy with interferon-α/ribavirin (Felger et al., 2013).

The main purpose of this preliminary study was to investigate the parameters of tryptophan and phenylalanine metabolism and in particular the role of immune-mediated tryptophan breakdown during the first months of alcohol abstinence in post-withdrawal alcohol-dependent patients. Additionally, the study aimed to examine the relationships between biochemical parameters and neuropsychiatric symptoms, which are manifest in this group of patients.
METHODS

Patients and treatment regime

Patients admitted for 8-week post-withdrawal in-patient alcohol rehabilitation treatment have been screened for this study. The treatment is based on a multimodal rehabilitation programme which includes medical management (somatic and psychiatric), psychotherapy (group and individual), physiotherapy, psychoeducation, psychosocial training and individual moderate physical exercise regimes. Only patients who completed alcohol withdrawal treatment and were motivated to maintain abstinence were admitted to the rehabilitation unit. To ensure alcohol abstinence during the treatment breath alcohol analysis and urine ethyl glucuronide as well as carbohydrate-deficient transferrin (CDT) and liver function parameters (alanine aminotransferase—ALAT, aspartate aminotransferase—ASAT, γ-glutamyl transferase—GGT) were acquired routinely.

Inclusion criteria

The following inclusion criteria have been applied: (a) at least 2 years of existing alcohol dependence as diagnosed according to the International Classification of Diseases (ICD), ICD-10; (b) average daily alcohol consumption: men—above 90 g/day, women—above 60 g/day; (c) age between 18 and 75 years; (d) motivation for an abstinence-oriented treatment; (e) informed consent.

Exclusion criteria

Patients with acute or exacerbated psychiatric condition or severe cognitive impairment, which precluded participation in the post-withdrawal treatment programme, as well as known chronic severe immune disease, cancer or pregnancy.

Study design

Clinical investigations and ratings as well as acquisition of blood samples were performed twice during the 8-week treatment: in the first week after the admission (4th week of abstinence in average) and in the eighth week of the treatment (12th week of abstinence) prior to the discharge.

Clinical assessments

The study-related investigation procedures were embedded in a semi-structured routine clinical psychiatric investigation. After given an informed consent patients have been investigated using standard clinical instruments. The severity of alcohol dependence was assessed using Alcohol Use Disorder Identification Test (AUDIT) (Saunders et al., 1993). Detailed data upon alcohol consumption in the last 3 months have been collected by means of Timeline Follow Back scale (TLFB) (Sobell and Sobell, 1992). Symptoms related to alcohol craving were collected by means of the German version of Obsessive-Compulsive Drinking Scale (OCDS-G) (Mann and Ackermann, 2000). Clinical symptoms of depression were rated by means of Montgomery-Asberg Depression Scale (MADRS) (Schmidtke et al., 1988). Additionally, Beck Depression Inventory (BDI) was applied as a self-rating instrument for various depressive symptoms (Hautzinger, 2002). BDI was selected for this study also because it can be used to collect symptoms associated with increased stress sensitivity. The patients were instructed to fulfil the self-rating scales and were offered assistance if needed.

Acquisition of blood samples and biological assays

Fasting blood samples were collected by trained clinic personnel between 6.00 a.m. and 7.30 a.m. and transported to the laboratory facility. Serum extracts were stored at −20°C until thawed for biological assays. Tryptophan and kynurenine concentrations (Widner et al., 1997) as well as concentrations of phenylalanine and tyrosine were determined by high-performance-liquid-chromatography utilizing the natural fluorescence of tryptophan (285 nm excitation and 360 nm emission wavelengths) and of phenylalanine and tyrosine (210 nm excitation and 302 nm emission wavelengths) as described (Neurauter et al., 2013). Kynurenine concentrations were measured by ultraviolet absorption at 360 nm (Widner et al., 1997). 25 ml of 2 M trichloroacetic acid was used to precipitate and separate proteins, and 3-nitro-l-tyrosine was used as an internal standard. The ratios of kynurenine to tryptophan (Kyn/Trp) concentrations and phenylalanine to tyrosine (Phe/Tyr) concentrations were calculated as indexes of tryptophan breakdown and phenylalanine 4-hydroxylase (PAH) activity, respectively. Concentrations of neopterin were measured by enzyme-linked immunosorbent assay (BRAHMS GmbH, Hennigsdorf, Germany) with a detection limit of 2 nM (Mayersbach et al., 1994). The stable NO metabolite nitrite (NO2) was determined in the serum specimens by the Griess reaction assay (Promega) (Capuron et al., 2011).

The Ethics Committee of Medical University Innsbruck approved the study protocol (AN 4400).

STATISTICAL METHODS

All statistical analyses were performed with SPSS, version 20. Prior to the analysis, laboratory parameters and clinical scales were scrutinized for normality by means of the Shapiro–Wilks test and by checking the skewness of the distribution (values >1 or <−1 were considered a violation of the normality assumption). As the majority of these variables proved to be non-normally distributed, non-parametric statistical tests were used throughout the analysis. The Wilcoxon signed-rank test was applied for pre-post comparisons of laboratory parameters and clinical variables (Week 1 vs. Week 8 of the study). Correlation analyses were performed using the Spearman rank correlation coefficient.

RESULTS

Demographics and alcohol-related parameters

Fifty-four patients (40 males, 14 females) completed the study during their 8-week post-withdrawal treatment. The characteristics of the patients as well as crucial parameters of the alcohol consumption prior to admission are presented in Table 1.

Clinical symptoms and laboratory parameters

Clinical assessments and self-report scales showed mild depressive symptoms and increased craving scores at the
admission (see Table 2). In accordance with improvement of clinical conditions during the abstinence both depression and craving scores decreased significantly during the 8 weeks of the treatment as measured by BDI, MADRS and OCDS (all $P<0.001$). Alcohol abstinence during the treatment is reflected by a substantial decrease of ASAT, GGT and carbohydrate-deficient transferrin (CDT) during the 8 weeks of the study (Table 2).

**Biochemical parameters**

There were significant increases of nitrite, kynurenine, Kyn/Trp between the first and eighth weeks of treatment (Table 2). Both phenylalanine and tyrosine concentrations show statistically significant decrease between the Weeks 1 and 8. The decrease of Phe/Tyr did not reach statistical significance. A slight decrease of neopterin and tryptophan was not statistically significant (Table 2).

In the first week of the study there was a significant correlation between neopterin and Kyn/Trp (Spearman $r$ ($r_S = 0.378$, $P < 0.01$). This correlation disappeared in the 8th week of the study (11th week of alcohol abstinence in average). In this study, neopterin concentrations correlated significantly with C-reactive protein (CRP) at Week 1 ($r_S = 0.378$, $P < 0.01$) but not at Week 8 of the study ($r_S = 0.011$, not significant). Tyrosine, phenylalanine, Phe/Tyr and nitrite concentrations showed no significant correlations with neopterin levels at any time of the study.

Neither total BDI score nor total OCDS score nor total MADRS score correlated significantly with biochemical parameters. Three BDI items—’irritability’ ($r_S = 0.293$, $P = 0.039$), ‘disturbed sleep’ ($r_S = 0.298$, $P = 0.036$) and ‘fatigue’ ($r_S = 0.288$, $P = 0.043$) and their sum score ($r_S = 0.343$, $P = 0.015$) correlated significantly with Kyn/Trp in the 8th week of the study.

**DISCUSSION**

This study investigated changes of an immune marker neopterin and selected amino acids during the medium-term post-withdrawal period in abstinent alcohol-dependent patients. Our data show constant tryptophan (Trp) concentrations and an increase of kynurenine (Kyn) and kynurenine to tryptophan ratio (Kyn/Trp) between Weeks 4 and 12 of alcohol abstinence and a correlation between neopterin and Kyn/Trp ratio in the first study visit (4th week of abstinence). Positive correlation between neopterin and Kyn/Trp has been previously shown to be a reliable marker of immune-mediated IDO activation (Schröcksnadel et al., 2006). Our findings do not provide evidence for the IDO activation during the whole study period but point towards a possible activation of the IDO in the very early stages of alcohol withdrawal. This would be in line with previous suggestions that chronic excessive alcohol consumption leads to activation of the immune system (Kelley and Danzter, 2011).

Immune activation in alcoholism involves several mechanisms. During an excessive consumption, alcohol and its...
metabolites initiate inflammatory cascades by increasing intestinal permeability for microbial agents, increasing the sensitivity of immune cells to stimulation and activation of innate immune pathways, whereas certain liver cells seem to contribute to the immune response (Wang et al., 2012). Patients recruited for our study completed alcohol withdrawal in average 4 weeks prior to the inclusion and did not show overt signs of alcoholic liver disease. Nevertheless, at the first study visit (4th post-withdrawal week) they showed slightly increased neopterin concentrations when compared with reference values of 100 healthy subjects (Geisler et al., 2014). This suggests a moderate immune activation without signs of overt liver disease.

Immune-mediated IDO activation has been previously shown to be responsible for depressive symptoms in different clinical conditions but the mechanisms are still not clarified (Oxenkrug, 2010; Haroon et al., 2012). Recent studies have shown that not just the decrease of tryptophan but also increase of kynurenine and Kyn/Trp correlate with the behavioural symptoms in patients receiving interferon-alpha immunotherapy (Bonaccorso et al., 2002; Capuron et al., 2003; Raison et al., 2009). Our data show a significant increase of Kyn (by about 23%) after 8 weeks but no signs of tryptophan depletion. The increase of kynurenine in the absence of any correlation between neopterin and Kyn/Trp at the Week 8 of the study (Week 12 of alcohol abstinence) suggests enhanced activity of TDO. At Week 8 of the study it appears that tryptophan is mostly metabolized by TDO, the activity of which is sensitive to glucocorticoid levels and therefore likely mediated by stress. Although in this study we did not measure cortisol levels, it is known that abstinent alcohol patients in the medium-term post-withdrawal period show high basal levels of salivary cortisol and an additional, though moderate, increase of cortisol concentration as a response to moderate affective stress (Sinha et al., 2009).

Previous studies have found disturbances of tryptophan metabolism and their association with depression in alcoholics. Particularly, a decreased tryptophan ratio to other aminoacids competing with tryptophan for brain entry has been investigated (Branchev and Lieber, 1982; Friedman et al., 1988). It has been suggested that diminished supply of tryptophan would lead to serotonin deficiency and therefore contribute to depression in alcoholics (Branchev et al., 1984).

Our results did not show any correlation between tryptophan concentrations and behavioural symptoms but Kyn/Trp marginally correlated with some self-reported symptoms in the later stage of the post-withdrawal period (Week 12 of abstinence). The assessment was based on a self-report by means of Beck Depression Inventory. The BDI is an established screening instrument with an ample list of various symptoms broadly attributed to depression. BDI contains also symptoms related to the increased levels of stress. Interestingly, only symptoms consistent with high stress levels, such as ‘irritability’, ‘fatigue’ and ‘sleep disturbances’—but none of the symptoms associated with depression such as for example ‘sadness’, ‘pessimism’ or ‘guilt’ correlated with the increased Kyn/Trp in our study.

Several metabolites of kynurenine are neuroactive and can modulate glutamatergic N-methyl-D-aspartate (NMDA) receptor. For example, quinolinic acid and 3-hydroxy-α-kynurenine are supposed to exert neurotoxic effects due to agonism of the NMDA receptor while kynurenic acid acts as a glutamate antagonist (Oxenkrug, 2010; Vécsei et al., 2013). Intensified kynurenine production due to stress-related TDO activation may lead to higher concentrations of various kynurenine metabolites.

As our data suggest, Kyn/Trp imbalance persists over several weeks after withdrawal and may be partly responsible for symptoms such as irritability and sleep disturbances, and therefore it is not necessarily the absence of alcohol exposure itself, which is responsible for the development of these symptoms. Also compounds contained in alcoholic beverages like several antioxidants could be involved, because their immunomodulatory properties could interfere with cytokine signalling and thus down-stream biochemistry such as the influence on IDO (Jeny et al., 2011).

In an earlier study of elderly persons significant relationships between neuropsychiatric scores and phenylalanine, tyrosine and Phe/Tyr concentrations have been observed (Capuron et al., 2011). In that study, a tendency appeared to exist that tryptophan metabolic alterations were associated with the depressive symptoms (MADRS items) in the patients, whereas disturbed phenylalanine metabolism was more related to neurovegetative symptoms. However, in our study no such relationships between phenylalanine metabolism and any of the neuropsychiatric scores performed were observed. Also neopterin and nitrite concentrations appeared to be widely unrelated to the neuropsychiatric scores.

In our study, the Phe and Tyr concentrations reduced significantly between the 4th and 12th week of abstinence whereas the decrease of Phe/Tyr ratio did not reach statistical significance. We found no correlations between these two amino acids and the behavioural symptoms. The role of Phe and Tyr changes in the recovery from alcoholism is still unclear. Our data suggest that further studies on metabolism of these two catecholamine precursors may be of importance.

The interpretation of the results of this preliminary study is limited by several factors. First, the study begins at 4th week of withdrawal, well after its acute phase. Second, the study was focussed on interaction between the immune activation and tryptophan metabolism and did not included factors influencing the activity of TDO such as cortisol concentrations. Nevertheless, our data point towards the importance of further studies on mechanisms of recovery from alcoholism concerning metabolism of important neurotransmitter precursors and neuroactive kynurenine metabolites and their impact on behavioural symptoms in alcohol-dependent patients during medium-term and long-term alcohol withdrawal.

In sum, our study shows that alcohol-associated IDO activation seems to play a marginal role in tryptophan metabolism in the medium-term post-withdrawal stages (post-withdrawal Week 4 in this study). In contrast, the shift in tryptophan breakdown towards kynurenine synthesis persists up to Week 12 of abstinence in this study. The increase of Kyn/Trp correlates modestly with several behavioural symptoms broadly associated with high stress levels. Our findings point towards a possible connection between behavioural symptoms and kynurenine metabolism in post-withdrawal alcohol-dependent patients. The role of kynurenine metabolism in the alcoholism-related behavioural symptoms needs further investigation.

Acknowledgements — The authors are thankful to Dr E. Braidt, Dr C. Nowakowski and Mrs Andrea Danninger for their valuable help in the acquisition of the clinical data.
Tryptophan metabolism in alcoholism

Funding — This study was partly supported by the Project D-151810-011-013 of Medical University Innsbruck.

Conflict of interest statement. None declared.

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