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Tryptophan degradation in multiple trauma patients: survivors versus non-survivors

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Abstract

Immune dysfunction in trauma patients is associated with immune system activation and inflammation. Cytokine-inducible enzyme indoleamine 2,3-dioxygenase (IDO) initiates the degradation of the essential aromatic amino acid tryptophan via the kynurenine pathway and could contribute to deficient immune responsiveness. Activated IDO is indicated by an increased kynurenine to tryptophan ratio (kyn/trp). This study investigated whether tryptophan degradation is associated with outcome of patients post trauma.

Tryptophan and kynurenine concentrations were measured by HPLC in serum specimens of 15 patients post-trauma during 12-14 days of follow up. Of every patient up to 5 specimens within this observation period were included in this analysis, in total 69 specimens were available. For further comparisons, concentrations of immune activation marker neopterin were measured.

Compared to healthy controls, average kyn/trp and kynurenine were increased in patients, and tryptophan concentrations were decreased. During follow-up, increasing kyn/trp and kynurenine concentrations (all $p < 0.001$) were observed, the changes of tryptophan concentrations were not significant. Non-survivors presented with higher kyn/trp and with higher kynurenine concentrations than survivors. Kyn/trp correlated with neopterin ($r_s = 0.590$, $p < 0.001$) concentrations.

Data imply that increased tryptophan degradation in patients is due to activated IDO, which most likely represents a result of host defence response. Data support a possible role of IDO in the development of immunodeficiency and death in patients.

Keywords: tryptophan; kynurenine; indoleamine (2,3)-dioxygenase; trauma; survival

Introduction

The inflammatory response in trauma patients is crucial for the fate of the patients. In such patients significant activation of various immune system compartments is demonstrated, which however is accompanied by diminished functional immune response and immune paralysis [1]. Enzyme indoleamine 2,3-dioxygenase (IDO) converts tryptophan to kynurenine and is strongly inducible by pro-inflammatory stimuli and preferentially by cytokine interferon- γ (IFN- γ) [2, 3]. Activated IDO diminishes the availability of the essential amino acid tryptophan and is considered as an immune defence mechanism, which suppresses the growth of intracellular bacteria, viruses and parasites like toxoplasma and of malignant tumor cells [3-5]. But also T-cell proliferation can be inhibited by IDO activity [6], and thus, immune response may be suppressed when tryptophan is diminished. In patients after multiple trauma, tryptophan deficiency was found to be associated with the decline of lymphocytes in numbers [7].

Pro-inflammatory stimuli like Th1-type cytokine IFN- γ induce IDO and neopterin production in human monocyte-derived macrophages and dendritic cells (DC) [8, 9]. In several groups of patients, e.g. suffering from virus infections, auto-immune syndromes and also arteriosclerosis and cancer, enhanced tryptophan degradation concurs with increased neopterin formation [10-13]. In trauma patients, plasma concentrations of neopterin are able to predict outcome [1, 14, 15]. From the data one can expect that in multiple trauma patients increased neopterin production will be paralleled by activation of IDO, which could represent a key to understand the negative contribution which immune activation and IFN- γ production might have in patients with trauma.

In this study, concentrations of tryptophan and kynurenine and kyn/trp were analyzed in patients with trauma and compared to the outcome. In addition, results on tryptophan metabolism were compared to concentrations of neopterin.

Materials and Methods

Patients

Fifteen patients post-trauma (12 males, 3 females; aged mean \pm S.D.: 40.4 \pm 17.2 years, range: 20 – 77 years; Apache score: 17.5 \pm 6.5 range: 8-34; Injury Severity Score (ISS): 39.1 \pm 13.1, range: 18-57) were included in this study who either were admitted to the Intensive Care Unit (ICU) of the Medical University of Vienna or to the ICU of Lorenz Boehler Trauma Center, Vienna (Table I). Inclusion criteria were age between 18 and 80 years and evidence of a multiple trauma (ISS >16). Exclusion criteria were known immunosuppressive therapy, known HIV infection or any other chronic disease. All patients were otherwise healthy before the trauma. Average length of stay in the ICU was 25.3 \pm 20.1 days. During follow up 6 patients died on days 7, 10, 14, 17, 26 and 37. Serum specimens were collected every third day during 12 – 14 days of follow up. For statistical analyses, specimens were divided into five groups: one specimen collected between days 1-2 of each patient was referred to group I, day 3-5 = group II, day 6-8 = group III, days 9-12 = group IV, and days 13-14 = group V. In total every patient contributed 3-5 specimens to the total number of 69 sera analyzed, which corresponds to 6 missing specimens over the whole period. All patients received standard parenteral nutrition (1700 kcal/d, 100g amino acids/d) after the end of hypodynamic shock. Samples of 49 healthy blood donors (21 women, 28 men, aged 35.2 \pm 13.5 years) served as a reference group [16].

The study was performed according to the Helsinki declaration. The protocol was approved by the local ethics committee, and written consent was granted by the next of

kin. The observed values of all study parameters had no influence whatsoever on the course of the therapy.

Measurements

Concentrations of tryptophan and kynurenine were determined by HPLC [16, 17], kyn/trp was calculated and expressed as μmol kynurenine per millimol tryptophan ($\mu\text{M}/\text{mM}$). Concentrations of Neopterin (BRAHMS, Hennigsdorf/Berlin, Germany; detection limit of 2 nM) were measured by commercially available ELISA according to the manufacturer's instructions.

Statistical Analysis

Demographic parameters were compared using the Student's t-test or X^2 -test. Results of measurements were expressed as mean \pm SD. Because not all the data sets showed normal distribution, non-parametric statistics were applied for data analyses, Kruskal Wallis H-test was used to look for differences in the median values between the 5 time groups, Mann Whitney U-test was subsequently applied for direct comparisons between grouped data. Spearman's rank correlation coefficients (r_s) were calculated for regression analyses. Changes during follow-up were calculated by paired rank test. Repeated measure analysis of variance with 5 steps was calculated concerning the 5 days of measurement. The Statistical Package for the Social Sciences (version 14 SPSS, Chicago, IL, USA) was used. P-values below 0.05 were considered to indicate significant differences.

Results

Kyn/trp and concentrations of kynurenine were increased and concentrations of tryptophan were decreased in patients compared to the reference group (gray boxes) (Fig. 1). None of the parameters correlated with Apache scores of patients. During follow-up, significant changes of kyn/trp ($H = 21.3$, $p < 0.001$) and kynurenine concentrations ($H = 21.2$, $p < 0.001$) were observed (Fig. 1). Kyn/trp and kynurenine concentrations increased from group I to II and remained higher than baseline throughout the further follow-up period (kyn/trp - I vs. II: $p < 0.01$, vs. III: $p < 0.01$, vs. IV: $p < 0.01$, vs. V: $p < 0.06$; kynurenine - I vs. II: $p < 0.01$, vs. III: $p < 0.01$, vs. IV: $p < 0.01$, vs. V: $p = 0.06$). Concentrations of tryptophan did not significantly differ from day I throughout the study period.

Patients who died during follow-up presented with significantly higher kyn/trp ($p < 0.05$, in groups II, IV and V; Fig. 1) and higher kynurenine concentrations ($p < 0.05$, group IV). Tryptophan concentrations were lower in non-survivors compared to survivors but did not differ significantly. When solely the concentrations measured in non-survivors were compared to baseline, the increases of kyn/trp and kynurenine became significant in group III ($p < 0.05$), the decline of tryptophan became nearly significant in group V ($U = 1.82$, $p = 0.068$). Repeated measure analysis of variance showed a significant difference between survivors and non-survivors concerning kynurenine concentrations ($p < 0.05$) and kyn/trp ($p < 0.01$).

Average concentrations of the additionally measured marker neopterin (mean \pm SD: 19.7 ± 21.7 nM) were increased in patients compared to the reference group. Patients who died during follow-up had higher neopterin ($p < 0.05$, groups II and III; $p < 0.01$, groups IV and V) concentrations compared to survivors. In the whole data set, there existed a positive correlation between kyn/trp and neopterin ($r_s = 0.590$, $p < 0.001$; data not shown).

Discussion

This study shows that tryptophan metabolism is altered in patients suffering from trauma compared to healthy controls. Compared to healthy controls, kynurenine concentrations and kyn/trp were elevated in patients, whereas tryptophan concentrations were decreased. During follow-up, a higher rate of kynurenine accumulation and of tryptophan degradation as indicated by higher kyn/trp was found in non-surviving trauma patients compared to survivors. This data confirm and extend earlier observations on enhanced degradation of tryptophan by IDO in an independent set of patients after major trauma [7].

In parallel to the increase of kynurenine concentrations and of kyn/trp, an increase of neopterin formation was observed. Because increase of neopterin is associated with Th1-type cytokines as IFN- γ [18], which induces both, tryptophan degradation via IDO and neopterin production in parallel [8, 11], we assume that accelerated tryptophan degradation is due to enhanced IDO activity in the patients. Low tryptophan concentrations are unlikely to be related to a reduced dietary intake of this essential amino acid, because in this case also a decrease of kynurenine and no change of kyn/trp would be expected. Increased IDO activity may indeed account for lowered tryptophan concentrations, and because of the significant association between neopterin concentrations and kyn/trp, also tryptophan degradation in our patients seems likely to be induced by pro-inflammatory stimuli, of which IFN- γ is the strongest inducer of IDO in macrophages and DC.

Measurement of circulating IFN- γ concentrations in serum/plasma of patients is usually very insensitive. That is why we refrained from analyzing IFN- γ concentrations in our patients. Like other cytokines, IFN- γ rapidly sticks to its specific receptors on target cells or their shed soluble forms and the therefore low concentrations in the blood are limiting the diagnostic application.

Post trauma, immunocompetent cells appear to respond against non-self structures, and cells produce cytokines including IFN- γ aimed at halting growth of pathogens. Activation of IDO as one out of several important antimicrobial mechanisms triggered by IFN- γ however not only affects microbes, it potentially may also counteract growth and development of T-cells [6]. A kind of equilibrium might develop between the activation degree of immunocompetent cells, their suppressive effect on microbes and also on themselves. In some patients the bactericidal effect of IDO will be able to stop the infectious process, in others immunocompetent cells are suffering from IDO activity more than the microbes, and then depletion of T-cells and immunosuppression is a consequence of the host's immune response against invading pathogens [19, 20]. Certainly this study is too preliminary to give a final answer to this question.

A similar relationship between enhanced tryptophan degradation and immune activation was found earlier in several other chronic diseases, like HIV-1 infection or malignancy [11, 21-24]. Moreover, in patients with HIV-1 infection and with cancer, enhanced tryptophan degradation and increased neopterin production were found to strongly predict shortened survival [21-23]. Also in patients with HIV-1 infection, antimicrobial/antiviral activity of IDO is suggested to contribute to immunodeficiency [24, 25] and may explain why immune activation markers are strong predictors of outcome. From *in vitro* studies it was concluded that not only the decline of tryptophan concentrations but also the increase formation of toxic tryptophan catabolites could be involved in the development of T-cell unresponsiveness [26]. Interestingly in our study, only the increase of kynurenine concentrations but not the decline of tryptophan was significantly associated with the outcome of the patients. Thus, data may favour a role of tryptophan catabolites rather than tryptophan lowering in the immune deviations which might be of relevance for the fatal outcome of some of our patients. Recently Pellegrin et al. showed that a decline of tryptophan was associated with the drop of T-lymphocytes in

patients after trauma [7]. In our study, the decrease of tryptophan concentrations did not reach the level of significance. Standard parenteral nutrition regimen which contains tryptophan as well as other essential amino acids was initiated early in our patients. It may have counteracted to some degree the loss of tryptophan despite further accelerating degradation of the amino acid and further increase of kynurenine concentrations.

Tryptophan deficiency resulting from its accelerated catabolism could also be involved in the increased risk for developing anemia, cachexia and neuropsychiatric abnormalities [11, 27, 28]. Still, the relevance of this assumption needs to be clarified in further extended studies.

In conclusion, accelerated tryptophan degradation is found in non-surviving patients post trauma. The same was true for higher concentrations of neopterin, which confirms and extends earlier data [14, 15]. Tryptophan degradation may represent one important aspect in the development of the post traumatic failure of the immune system to respond appropriately. More extended and follow-up studies examining the impact of tryptophan degradation and its relationship with cellular immune activation in patients suffering from trauma may provide interesting new data, which should be helpful to define new therapeutic intervention strategies. Larger clinical studies are necessary to find out more about the potential prognostic expressiveness of tryptophan metabolism in patients suffering from trauma.

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References

1. Zedler, S. and Faist, E. (2006) The impact of endogenous triggers on trauma-associated inflammation. *Curr. Opin. Crit. Care* **12**, 595-601.
2. Taylor, M.W. and Feng, G.S. (1991) Relationship between interferon-gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism. *FASEB J.* **5**, 2516-2522.
3. Brown, R.R., Ozaki, Y., Datta, S.P., Borden, E.C., Sondel, P.M. and Malone, D.G. (1991) Implications of interferon-induced tryptophan catabolism in cancer, auto-immune diseases and AIDS. *Adv. Exp. Med. Biol.* **294**, 425-435.
4. Pfefferkorn, E.R. (1984) Interferon gamma blocks the growth of *Toxoplasma gondii* in human fibroblasts by inducing the host cells to degrade tryptophan. *Proc. Natl. Acad. Sci. U.S.A.* **81**, 908-912.
5. Carlin, J.M., Ozaki, Y., Byrne, G.I., Brown, R.R. and Borden, E.C. (1989) Interferons and indoleamine 2,3-dioxygenase: role in antimicrobial and antitumor effects. *Experientia* **45**, 535-541.
6. Mellor, A.L. and Munn, D.H. (1999) Tryptophan catabolism and T-cell tolerance: Immunosuppression by starvation? *Immunol. Today* **20**, 469-473.
7. Pellegrin, K., Neurauter, G., Wirleitner, B., Fleming, A.W., Peterson, V.M. and Fuchs, D. (2005) Enhanced enzymatic degradation of tryptophan by indoleamine 2,3-dioxygenase contributes to the tryptophan-deficient state seen after major trauma. *Shock* **23**, 209-215.
8. Weiss, G., Murr, C., Zoller, H., et al. (1999) Modulation of neopterin formation and tryptophan degradation by Th1- and Th2-derived cytokines in human monocytic cells. *Clin. Exp. Immunol.* **116**, 435-440.
9. Wirleitner, B., Reider, D., Ebner, S. et al. (2002) Monocyte-derived dendritic cells release neopterin. *J. Leukoc. Biol.* **72**, 1148-1153.
10. Fuchs, D., Moeller, A.A., Reibnegger, G., et al. (1991) Increased endogenous interferon-gamma and neopterin correlate with increased degradation of tryptophan in human immunodeficiency virus type 1 infection. *Immunol. Lett.* **28**, 207-211.
11. Wirleitner, B., Neurauter, G., Schröcksnadel, K., Frick, B. and Fuchs, D. (2003) Interferon- γ -induced conversion of tryptophan: immunologic and neuropsychiatric aspects. *Curr. Med. Chem.* **10**, 1581-591.
12. Pertovaara, M., Raitala, A., Juonala, M., et al. (2007) Indoleamine 2,3-dioxygenase enzyme activity correlates with risk factors for atherosclerosis: the Cardiovascular Risk in Young Finns Study. *Clin. Exp. Immunol.* **148**, 106-111.
13. Pertovaara, M., Raitala, A., Uusitalo, H., et al. (2005) Mechanisms dependent on tryptophan catabolism regulate immune responses in primary Sjogren's syndrome. *Clin. Exp. Immunol.* **142**, 155-161.
14. Strohmaier, W., Redl, H., Schlag, G. and Inthorn, D. (1987) D-erythro-neopterin plasma levels in intensive care patients with and without septic complications. *Crit. Care Med.* **15**, 757-760.
15. Hensler, T., Sauerland, S., Lefering, R., et al. (2003) The clinical value of procalcitonin and neopterin in predicting sepsis and organ failure after major trauma. *Shock* **20**, 420-426.
16. Widner, B., Werner, E.R., Schennach, H., Wachter, H. and Fuchs, D. (1997) Simultaneous measurement of serum tryptophan and kynurenine by HPLC. *Clin. Chem.* **43**, 2424-2426.
17. Laich, A., Neurauter, G., Widner, B. and Fuchs, D. (2002) More rapid method for simultaneous measurement of tryptophan and kynurenine by HPLC. *Clin. Chem.* **48**, 579-581.

18. Murr, C., Widner, B., Wirleitner, B. and Fuchs, D. (2002) Neopterin as a marker for immune system activation. *Curr. Drug Metabol.* **3**, 175-187.
19. Wood, K.J. and Sawitzki, B. (2006) Interferon gamma: a crucial role in the function of induced regulatory T cells in vivo. *Trends Immunol.* **27**, 183-187.
20. Schroecksnadel, K. and Fuchs, D. (2006) Interferon- γ for counteracting T-cell activation. *Trends Immunol.* **27**, 398.
21. Giusti, R.M., Maloney, E.M., Hanchard, B., et al. (1996) Differential patterns of serum biomarkers of immune activation in human T-cell lymphotropic virus type I-associated myelopathy/tropical spastic paraparesis and adult T-cell leukemia/ lymphoma. *Cancer Epidemiol. Biomarkers Prevent.* **5**, 699-709.
22. Brandacher, G., Perathoner, A., Ladurner, R., et al. (2006) Prognostic value of indoleamine 2,3-dioxygenase expression in colorectal cancer: effect on tumor-infiltrating T cells. *Clin. Cancer Res.* **12**, 1144-1151.
23. Weinlich, G., Murr, C., Richardsen, L., Winkler, C. and Fuchs, D. (2007) Decreased serum tryptophan concentration predicts poor prognosis in malignant melanoma patients. *Dermatology* **214**, 8-14.
24. Boasso, A. and Shearer, G.M. (2007) How does indoleamine 2,3-dioxygenase contribute to HIV-mediated immune dysregulation. *Curr. Drug Metab.* **8**, 217-223.
25. Schroecksnadel, K., Zangerle, R., Bellmann-Weiler, R., Garimorth, K., Weiss, G. and Fuchs, D. (2007) Indoleamine-2,3-dioxygenase and other interferon- γ -mediated pathways in patients with human immunodeficiency virus infection. *Curr. Drug Metab.* **8**, 225-236.
26. Terness, P., Bauer, T.M., Rose, L., et al. (2002) Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: mediation of suppression by tryptophan metabolites. *J. Exp. Med.* **196**, 447-457.
27. Widner, B., Laich, A., Sperner-Unterweger, B., Ledochowski, M. and Fuchs, D. (2002) Neopterin production tryptophan degradation and mental depression: what is the link? *Brain Behav. Immun.* **16**, 590-595.
28. Myint, A.M., Kim, Y.K., Verkerk, R., Scharpe, S., Steinbusch, H. and Leonard, B. (2007) Kynurenine pathway in major depression: evidence of impaired neuroprotection. *J. Affect. Disord.* **98**, 143-151.

Table I. Characteristics of all patients, survivors versus non-survivors. Data are presented as mean values \pm SD if not stated otherwise (*Student's t-test, #Pearson's chi-square test, all not significant).

	Survivors	Non-Survivors	p-value
Number of patients	9	6	
Age	34.3 \pm 10.4	50.3. \pm 21.8	0.141*
Gender (female/male)	2/7	1/5	0.605#
Length of stay in ICU (days)	32.9 \pm 21.3	14.0 \pm 12.1	0.137*
Received inotropes (yes/no)	8/1	6/0	0.412#
Apache score	15.0 \pm 4.7	20.8 \pm 7.3	0.154*
Injury Severity Score (ISS)	35.2 \pm 10.9	44.8 \pm 14.9	0.170*

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LEGENDS TO THE FIGURES

Fig. 1 Serum kynurenine to tryptophan ratio (upper), kynurenine (middle) and tryptophan (lower) concentrations in plasma samples of 15 patients post trauma during follow-up for 2 weeks (9 survivors, indicated by straight lines, black box with white cross indicating mean values; 6 non-survivors, dotted lines, black triangle indicating mean values; black circles indicating mean of all patients; gray fields in the background showing control values of a reference group, mean \pm SD; numbers of subjects (survivors/non-survivors) in bottom line; *p <0.05 survivors compared to non-survivors)

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