Original Article

Activation of Indoleamine 2,3-Dioxygenase in Children with Acute Febrile Diseases

JMAJ 48(6): 277–282, 2005

Mio Sakuma,[*1,2] Yasutaka Mizuno,[*1] Hironori Nakamura,[*1] Mitsuyoshi Urashima[*1,2]

Abstract

Background The clinical severity of acute inflammatory diseases may be worsened by tissue injury triggered by oxidative stress. Indoleamine 2,3-dioxygenase (IDO), which catabolizes tryptophan (TRP) to kynurenine (KYN), is known to be an antioxidant, but its kinetics during the acute febrile phase in children have not been delineated.

Methods We retrospectively measured serum TRP, KYN, and vitamin A/E concentrations as well as C-reactive protein (CRP) levels in 89 children with acute febrile disease (n = 62) and afebrile chronic disease (n = 27). Next, the protective effects of vitamin A on IDO activity were studied using phytohemagglutinin (PHA)-stimulated human mononuclear cells (MCs) in vitro.

Results Serum levels of TRP and the ratio of TRP/KYN were significantly lower in febrile children than in afebrile children ($P<0.0001$) and in PHA-stimulated MCs than non-stimulated cells ($P<0.0001$). There was positive linear relationship between serum levels of vitamin A and TRP ($P<0.001$) in children who were CRP positive (CRP >= 0.5 mg/dL). However, changes in TRP/KYN triggered by PHA were not altered by the addition of vitamin A.

Conclusions These results suggest IDO activity may be stimulated in children with acute febrile diseases.

Key words Tryptophan, Kynurenine, IDO, Infectious disease, Children

Introduction

Oxidants produced during inflammation contribute to cell and tissue damage either directly or through the activation of proteases.[1] For example, influenza virus triggers an oxidant stress response in the lungs that induces pneumonia.[2] However, free radicals at the sites of inflammation can be removed by antioxidant defenses, including catalase, glutathione peroxidase, superoxide dismutase, and vitamins A, C, E as well as reactive oxygen species including nitric oxide and related species.[3,4] Specifically, antioxidants have been shown to reduce the severity of asthma by inhibiting tissue damage.[5] In addition, plasma levels of specific carotenoids have been shown to be significantly lower in children with cystic fibrosis than in normal subjects,[6] which suggests that antioxidants may be consumed, which destroy toxic oxygen species in inflammatory states.

In particular, treatment with vitamin A reduces morbidity and mortality associated with measles independent of nutritional

*1 Department of Pediatrics, Jikei University School of Medicine, Tokyo
*2 Division of Clinical Research and Development, Jikei University School of Medicine, Tokyo
Correspondence to: Mitsuyoshi Urashima MD, PhD, MPH, Division of Clinical Research and Development, Jikei University School of Medicine, 3-25-8 Nishi-shimbashi, Minato-ku, Tokyo 105-8461, Japan. Tel: 81-3-3433-1111, Fax: 81-3-5400-1250, E-mail: urashima@jikei.ac.jp

deficiency.[7] In addition, in areas where vitamin A deficiency and malnutrition are documented public health problems, the regular provision of vitamin A supplements to children, at a level potentially obtainable from foods, was shown to contribute substantially to children's survival: overall mortality was reduced on average by 54%.[8] Thus, at least in part, supplementation with vitamin A and related products may play an important role in reducing complications from infectious diseases in children by acting as an antioxidant. However, the kinetics of vitamin A during the acute phase of common infectious diseases remains uncertain in developed countries.

In addition to vitamin A, indoleamine 2,3-dioxygenase (IDO), a rate-limiting enzyme that catabolizes tryptophan (TRP) to kynurenine (KYN),[9] works as one of the major antioxidants preventing oxidative damage to host tissue during inflammation.[10,11] The activity of IDO apparently increases more than 100-fold in the lungs of mice infected with the influenza virus.[12,13] However, the metabolism of TRP in relation to IDO activity remains uncertain in the serum of patients in the acute phase of an infectious disease.

In this study, we retrospectively measured the concentrations of TRP/KYN to study the enzymatic activity of IDO in serum from children with a variety of acute or chronic pediatric diseases, and compared findings between children in febrile and afebrile states. Moreover, associations between TRP/KYN and vitamin A were examined in vivo and in vitro.

Subjects and Methods

Population

This study was approved by institutional review board of Yokohama General Hospital. A total of 146 children visited a community hospital between September 2002 and December 2002 and underwent blood tests including C-reactive protein (CRP) for clinical purposes. Of these, 57 children were excluded from this study because serum samples were not kept. The present study therefore retrospectively examined extra volume serum samples derived from 89 patients. The patients were categorized as febrile when they complained of fever of more than 38.0 centigrade.

Preparation of blood-derived mononuclear cells for in vitro experiment

Heparinized peripheral blood (50 mL) was obtained from five independent healthy adult volunteers. Mononuclear cells (MCs) were isolated from the blood by centrifugation on a Ficoll-Paque density gradient (Amersham Biosciences, Uppsala, Sweden). Cells were cultured for 14 days in 90% RPMI1640 with L-glutamine/streptomycin-penicillin and 10% fetal bovine serum at 37°C in a humidified 5% CO_2 atmosphere at 1.0×10^6/mL in 1 mL, with or without phytohemagglutinin (PHA) (1 mg/mL) (Sigma Chemical Co. Ltd., St. Louis, MO, USA), with or without vitamin A (25, 50, 100 μg/dL) (Sigma Chemical Co. Ltd.) plus vitamin A binding protein (1 mg/mL) (Sigma Chemical Co. Ltd.).

High-pressure liquid chromatography (HPLC) determination of TRP/KYN and vitamin A/E

Total free TRP and KYN were quantified in serum or culture media by HPLC as previously described.[14] Similarly, serum levels of vitamin A and vitamin E were measured with HPLC.[15]

Statistical analysis

Levels of TRP and KYN, the ratio of TRP/KYN, as well as vitamin A and vitamin E levels were compared between afebrile and febrile patients using the Student's t-test. Linear regression analysis was performed to determine any association between the two parameters. A p-value of 0.05 was considered significant. All tests were performed using STATA 8.0 software (STATA Corpo-

ration, College Station, TX, USA).

Results

Patients' characteristics

Of the 89 children included in the study, 51 were male and 38 were female. The mean age of patients was 5.3±4.6 years. Sixty-two patients were in a febrile condition due to an infectious disease, including antigen-confirmed influenza (n=11); croup, bronchitis, bronchiolitis, or pneumonia (n=24); viral gastroenteritis (n=10); measles or mumps (n=4); other conditions (n=13). Twenty-seven patients were afebrile but had non-infectious diseases, including bronchial asthma and/or atopic dermatitis (n=9); epilepsy (n=3); liver dysfunction (n=2); other conditions (n=13).

Differences in serum measures between afebrile and febrile patients

The means±standard deviations for the various variables measured in the 89 patients were as follows: TRP=35.3±11.2μM; KYN=2.3±0.9μM; ratio of TRP/KYN =17.8±8.3; vitamin A=20.9±10.8μg/dL; vitamin E=6.9±2.0μg/dL; CRP=1.3±2.5 mg/dL. Serum levels of TRP and the ratio of TRP/KYN were significantly lower in febrile children than in afebrile children (P<0.0001), whereas KYN levels were not significantly different between the two groups (Fig. 1).

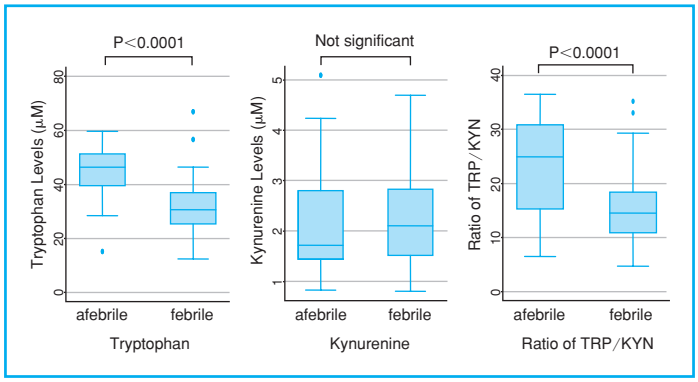

Fig. 1 Difference in serum levels of TRP, KYN and ratio of TRP/KYN between afebrile and febrile patients
Student's t-test was performed and p-values are shown.

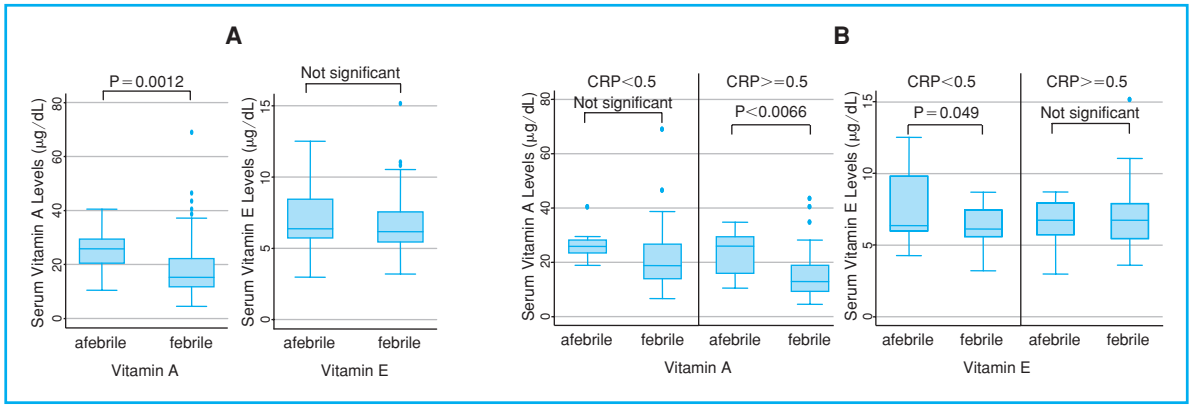

Fig. 2 Difference in serum levels of vitamin A and vitamin E between afebrile and febrile patients
Student's t-test was performed and p-values are shown.
(A) Not stratified by CRP, (B) Stratified by CRP: CRP was negative (<0.5 mg/dL); CRP positive (≥0.5 mg/dL).

Serum levels of vitamin A were significantly lower in febrile children than in afebrile children (P = 0.012); however, vitamin E levels were not significantly different between the two groups (Fig. 2A). Patients were then further stratified by whether they had negative or positive CRP levels (CRP negative, <0.5 mg/dL; CRP positive, >= 0.5 mg/dL) (Fig. 2B). Levels of vitamin A significantly decreased in febrile children who were CRP positive (P = 0.0066). In contrast, levels of vitamin E significantly decreased in febrile children who were CRP negative (P = 0.049).

Associations between vitamin A and TRP/KYN levels

In children who were CRP positive, there was a positive linear association between serum levels of vitamin A and TRP (P<0.001); no significant association between these values was detected in children who were CRP negative (Fig. 3). Similarly, in children who were CRP positive, there was a positive linear association between serum levels of vitamin A and the ratio of TRP/

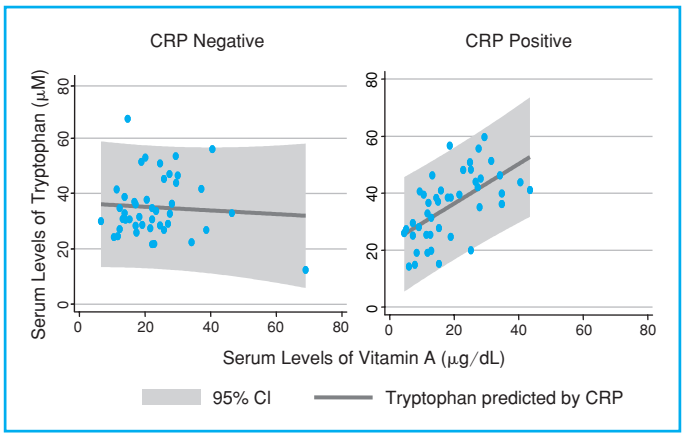

Fig. 3 Scattered plot showing association between serum levels of vitamin A and tryptophan stratified CRP
Dots: Observed. Line: Predicted value with 95% confidence intervals.

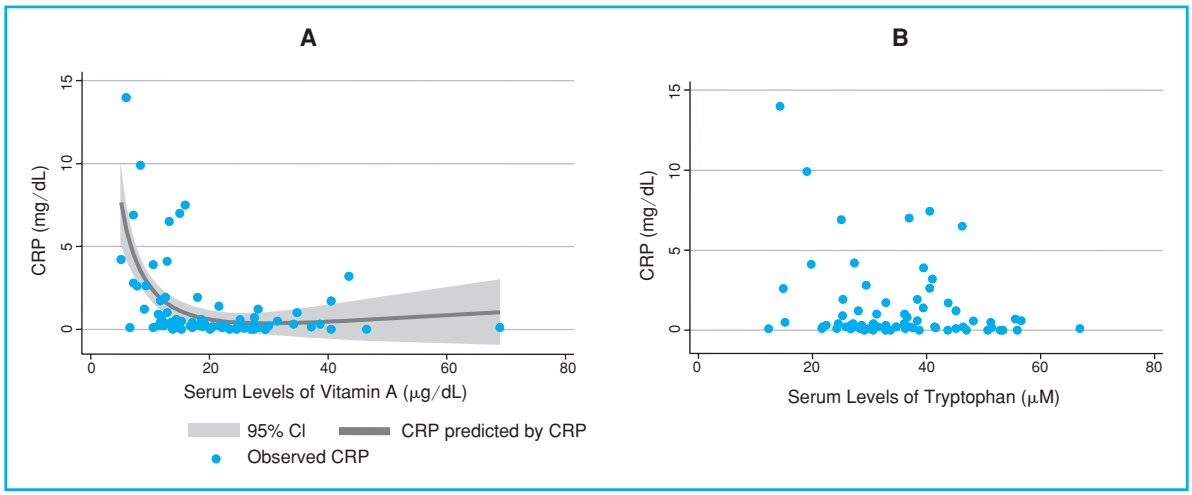

Fig. 4 (A) Scattered plot showing associations between serum levels of vitamin A and CRP
(B) Scattered plot showing associations between serum levels of tryptophan and CRP
Dots: Observed. Line: Predicted value with 95% confidence intervals.

KYN ($P=0.015$); no significant association was seen in children who were CRP negative. No significant associations between vitamin A and KYN were seen. Serum levels of vitamin E were not significantly associated with TRP or KYN levels or the ratio of TRP/KYN.

Associations between vitamin A and CRP levels

Serum vitamin A levels had a significant and inverse association with CRP levels (Fig. 4A). Specifically, when vitamin A levels were above 20 μg/dL, no child had a CRP level higher than 4 mg/mL. In contrast, there was no significant association between TRP and CRP levels (Fig. 4B). Neither KYN nor the ratios of TRP/KYN were associated with CRP levels (data not shown).

Effects of PHA on metabolism of TRP and KYN and the ratio of TRP/KYN

Supernatants of mononuclear cells stimulated with PHA contained significantly lower TRP levels as well as a lower ratio of TRP/KYN and significantly higher KYN levels than non-stimulated cells, ($P<0.0001$) (Fig. 5). Levels of TRP, KYN, and the ratio of TRP/KYN were not altered by the addition of vitamin A and vitamin A binding protein (data not shown in Fig. 5).

Discussion

Serum levels of TRP and the ratio of TRP/KYN were significantly lower in febrile children than in afebrile children. Moreover, stimulation of mononuclear cells with PHA in vitro remarkably decreased TRP levels and the ratio of TRP/KYN while increasing KYN levels. These findings suggest that nonspecific stimulation of the immune system may facilitate metabolism of TRP to KYN, which correlates with activation of IDO both in vivo and in vitro. Increased concentrations of KYN have been reported during febrile convulsions,[16] suggesting that enhanced TRP metabolism during febrile conditions in children may trigger convulsions.

Levels of vitamin A significantly decreased in febrile children who were CRP positive; this decrease was not seen in children who were CRP negative. Previous reports have shown that serum retinol and retinol binding protein concentrations fall in periods of infection.[17] Similarly, levels of acute-phase proteins have been shown to have an inverse relationship with vitamin A concentrations in children.[18–20] These findings are consistent with our results. In children who were CRP positive, there was a positive linear association between serum levels of vitamin A and TRP as well as the ratio of TRP/KYN; how-

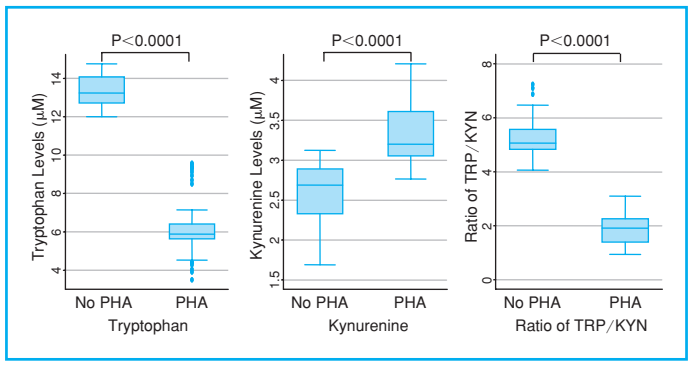

Fig. 5 Effects of PHA on metabolism of tryptophan, kynurenine and ratio of tryptophan/kynurenine
Mononuclear cells derived from five independent adults were cultured for 14 days in 90% RPMI1640 with 10% FBS at 37°C with or without PHA. Lower ratio of TRP/KYN suggests activation of IDO.

ever, no significant association between these variables was detected in children who were CRP negative. The addition of vitamin A did not affect the metabolism of TRP in this study. To our knowledge, there are no reports that describe the association between vitamin A and TRP metabolism. Our findings suggest the reduction of vitamin A levels and facilitation of IDO-related metabolism may be an independent phenomenon following pro- and inflammatory reactions.

These results suggest that IDO activity may be stimulated in children with acute febrile diseases.

Acknowledgements

The authors gratefully acknowledge Professor Yoshikatsu Eto, MD, Department of Pediatrics, The Jikei University School of Medicine, for critical reading of the manuscript.

References

1. Weiss SJ. Tissue destruction by neutrophils. N Engl J Med. 1989;320:365–376.
2. Choi AM, Knobil K, Otterbein SL, Eastman DA, Jacoby DB. Oxidant stress responses in influenza virus pneumonia: gene expression and transcription factor activation. Am J Physiol. 1996;271:L383–391.
3. Djordjevic VB. Free radicals in cell biology. Int Rev Cytol. 2004; 237:57–89.
4. Gerber M. Biofactors in the Mediterranean diet. Clin Chem Lab Med. 2003;41:999–1004.
5. Comhair SA, Bhathena PR, Dweik RA, Kavuru M, Erzurum SC. Rapid loss of superoxide dismutase activity during antigen-induced asthmatic response. Lancet. 2000;355:624.
6. Homnick DN, Cox JH, DeLoof MJ, Ringer TV. Carotenoid levels in normal children and in children with cystic fibrosis. J Pediatr. 1993;122:703–707.
7. Hussey GD, Klein M. A randomized, controlled trial of vitamin A in children with severe measles. N Engl J Med. 1990;323:160–164.
8. Rahmathullah L, Underwood BA, Thulasiraj RD, Milton RC, Ramaswamy K, Rahmathullah R, Babu G. Reduced mortality among children in southern India receiving a small weekly dose of vitamin A. N Engl J Med. 1990;323:929–935.
9. Munn DH, Sharma MD, Lee JR, Jhaver KG, Johnson TS, Keskin DB, Marshall B, Chandler P, Antonia SJ, Burgess R, Slingluff CL Jr, Mellor AL. Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. Science. 2002; 297:1867–1870.
10. Thomas SR, Stocker R. Redox reactions related to indoleamine 2,3-dioxygenase and tryptophan metabolism along the kynurenine pathway. Redox Rep. 1999;4:199–220.
11. Christen S, Peterhans E, Stocker R. Antioxidant activities of some tryptophan metabolites: possible implication for inflammatory diseases. Proc Natl Acad Sci USA. 1990;87:2506–2510.
12. Yoshida R, Urade Y, Tokuda M, Hayaishi O. Induction of indoleamine 2,3-dioxygenase in mouse lung during virus infection. Proc Natl Acad Sci USA. 1979;76:4084–4086.
13. Jacoby DB, Choi AM. Influenza virus induces expression of antioxidant genes in human epithelial cells. Free Radic Biol Med. 1994;16:821–824.
14. Frumento G, Rotondo R, Tonetti M, Damonte G, Benatti U, Ferrara GB. Tryptophan-derived catabolites are responsible for inhibition of T and natural killer cell proliferation induced by indoleamine 2,3-dioxygenase. J Exp Med. 2002;196:459–468.
15. Schulpis KH, Michalakakou K, Gavrili S, Karikas GA, Lazaropoulou C, et al. Maternal-neonatal retinol and alpha-tocopherol serum concentrations in Greeks and Albanians. Acta Paediatr. 2004;93:1075–1080.
16. McKiernan J, Mellor D, Court S, Edson J, Lacey K. Hydroxykynurenine/hydroxyanthranilic acid ratios and febrile convulsions. Arch Dis Child. 1980;55:873–875.
17. Tanumihardjo SA. Assessing vitamin A status: past, present and future. J Nutr. 2004;134:290S–293S.
18. Thurnham DI, McCabe GP, Northrop-Clewes CA, Nestel P. Effects of subclinical infection on plasma retinol concentrations and assessment of prevalence of vitamin A deficiency: meta-analysis. Lancet. 2003;362:2052–2058.
19. Stephensen CB, Franchi LM, Hernandez H, et al. Assessment of vitamin A status with the relative-dose-response test in Peruvian children recovering from pneumonia. Am J Clin Nutr. 2002;76: 1351–1357.
20. Stephensen CB, Gildengorin G. Serum retinol, the acute phase response, and the apparent misclassification of vitamin A status in the third National Health and Nutrition Examination Survey. Am J Clin Nutr. 2000;72:1170–1178.