Original article

Plasma amino acid levels in individuals with bacterial pneumonia and healthy controls

Hideki Ikeda*

Department of Pulmonary Medicine, Sanyudo Hospital, Yonezawa, Japan

SUMMARY

Background & aims: Amino acids play an important role in immune responses and as neurotransmitters. During the course of a bacterial pneumonia episode, from the onset to the recovery phase, immune responses dramatically change, as does the metabolism of amino acids, a concept referred to as immunonutrition. We investigated the differences in plasma amino acid levels (PAA) between the acute and recovery phases in individuals with community-acquired pneumonia (CAP) and healthy controls.

Methods: Two groups of participants were recruited: Healthy adults aged over 60 years and patients hospitalized with CAP. Samples were collected on Day 0 (the day of admission) and Day 7 (after 6–8 days treatment).

Results: A total of 93 healthy adults and 60 patients with CAP participated in the study. Of those with CAP, 43 had their amino acids measured on Day 7. Patients with CAP had markedly decreased PAA of 12 amino acids on Day 0. Citrulline, histidine, and tryptophan remained low in male, while aspartic acid, asparagine, ornithine, proline, and threonine were higher on Day 7 in both males and females. Phenylalanine increased at Day 0 and Day 7.

Conclusions: The findings suggest that the host response against bacterial infection changed the plasma amino acid levels. PAA on Day 7 (representing convalescence) continued to display an amino acid profile distinct from that observed in healthy individuals. Based on these findings, reconsideration for providing amino acids to patients with bacterial pneumonia should be needed depending on stage of the pneumonia from the perspective of immunonutrition.

© 2021 The Author(s). Published by Elsevier Ltd on behalf of European Society for Clinical Nutrition and Metabolism. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

In recent years, advances in analytical methodologies have paved the way for comprehensive detection of a wide range of chemical compounds; and metabolomics has allowed analysis of metabolites in various diseases [1]. Specifically, amino acids serve an important role in the immune response [2] and as neurotransmitters [3]. Both undernutrition and overnutrition can have a significant influence on the immune system, and the modulation of immune activity by intervention with specific nutrients is termed immunonutrition [4]. It has been reported that the reduction of plasma amino acids was associated with incidence of respiratory exacerbation [5]. The plasma levels of some amino acids, namely threonine, serine, asparagine, glutamine, citrulline, and histidine, have been reported to be markedly reduced in hospitalized patients with acute exacerbations of chronic obstructive pulmonary disease caused by bacterial infection [6]. Recent studies revealed the existence of microbiota in the lung under physiological condition, which form a mutualistic relationship with host [7]. In addition, competition with other bacteria for nutrient availability affects the growth of pathogens [8]. Amino acids are now recognized as mediators of metabolic cross talk between the host and pathogen [9]. However, there have been no detailed studies of the changes in plasma amino acid levels over the course of an episode of bacterial pneumonia in humans. The aim of this study was to describe changes in plasma amino acid levels in the acute and recovery phases of bacterial pneumonia in hospitalized patients comparing their amino acid profiles with healthy controls.

Abbreviations: CAP, community acquired pneumonia; PAA, plasma amino acid; LC-MS, liquid chromatography-mass spectrometry; Day 0, the day of admission; Day 7, after 6–8 days treatment; BMI, body mass index; NO, nitric oxide.

* Department of Pulmonary Medicine, Sanyudo Hospital, Chuo 6-1-219, Yonezawa, Yamagata 992-0045, Japan.

E-mail address: h-ikeda@js3.so-net.ne.jp.

https://doi.org/10.1016/j.clnesp.2021.06.021
2405-4577/© 2021 The Author(s). Published by Elsevier Ltd on behalf of European Society for Clinical Nutrition and Metabolism. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
2. Methods

2.1. Subjects and methods

The bacterial pneumonia group comprised patients admitted to our hospital between March 2016 and December 2019 who were diagnosed with community-acquired pneumonia (CAP), according to Japanese Respiratory Society guidelines [10] and the criteria in a previous study on CAP [11]. The diagnostic criteria were expectoration of purulent sputum [12], an increase in C-reactive protein, white blood cell count [13], the appearance of new opacities on thoracic radiography or computed tomography, and fever [14]. All these criteria were required to be met for a diagnosis of CAP. And the patients who already had medication with antibiotics within seven days or had any symptoms of CAP more than two days before admission were excluded. Therefore, we considered the period within 24 h after admission as the acute phase of CAP. The CAP severity was scored according to the A-DROP scoring system [10,15]. The A-DROP scoring system is a modified version of CURB-65 [16] proposed by the Japanese Respiratory Society. This scoring system is presented as a six-point scale (0–5) assessing the following parameters: (1) Age (male >70 years, female >75 years), (2) dehydration (+) or blood urea nitrogen >21 mg/dL, (3) PaO2 <60 torr (SpO2 ≤90), (4) confusion, and (5) systolic blood pressure <90 mmHg. Patients with diabetes (hemoglobin Alc (HbA1c) >6.1% or taking diabetes medication), chronic renal failure (estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m²), or a history of gastric resection were excluded. Sputum samples were scanned by Gram staining and cultured on chocolate agar with sheep blood, mannitol salt agar with egg yolk, and modified Drigalski agar.

Samples for acute phase of CAP were collected within 24 h after admission (Day 0) on all patients. Of the patients with CAP, some provided convalescent blood samples at 6–8 days post-treatment (Day 7). Those in the CAP group were unable to fast since pneumonia treatment was a priority; thus, Day 0 samples and Day 7 samples were collected 2–3 h after lunch. Meal plans were planned under the guidance of a nutritional manager to contain 600 kcal, 25 g protein, and 80–100 g carbohydrates. All meals consisted of common Japanese foodstuffs without nutritional intervention.

The control group comprised healthy individuals who received a health examination at our hospital between May 2018 and January 2019. The patients with CAP in this study were all aged >60 years with the exception of one male and one female, and the average age was 78.2 and 82.2 years for male and female, respectively. Therefor, participants for control were selected to ensure that each 5-year interval of age above 60 years included ten males and ten females. Both male and female CAP patients achieved A-DROP scores ranging from 0 to 3 with a median score of 2 (Table 2). No patients had very severe pneumonia with an A-DROP score of 4 or 5. The severity of pneumonia did not significantly differ between male and female. The medications for CAP group comprised antibiotics and electrolyte solution with glucose or lactic acid. No patients were medicated with metabolism regulators such as antidiabetic drugs or steroid hormones. All patients in the CAP group completed their antibiotic course of 7 days, and none experienced pneumonia recurrence. Blood samples for amino acid analysis on Day 7 were obtained from 26 males and 17 females.

The etiologic bacteria were identified by culture in 48 patients. The pathogens included Streptococcus pneumoniae (n = 13), Haemophilus influenzae (n = 11), Staphylococcus aureus (n = 6), Klebsiella pneumoniae (n = 4), and other bacteria (n = 14). The remaining 12 patients had either Gram-positive cocci (n = 8) or Gram-negative rods (n = 4) on microscopic analysis. There were no significant differences in the amino acid levels according to the Gram stain type.

In the control group, the levels of 17 amino acids were higher in males than in females (Table 3). Multiple regression analysis revealed that eight amino acids correlated with age and/or BMI. Two amino acids (arginine and cystine) in males and three amino acids (serine, threonine, and tyrosine) in females were correlated with age. Four amino acids (cystine, glutamic acid, isoleucine, and leucine) in males and two amino acids (leucine and tyrosine) in females were correlated with BMI. Therefore, these amino acid levels were adjusted by linear regression for age and BMI for comparisons between the control and CAP groups. However, this compensation affected only the statistical significance (p < 0.05) between the control and Day 7 amino acid levels (lysine in male and serine and tyrosine in female).

In both the male and female participants, 12 amino acids (alanine, arginine, asparagine, citrulline, glutamine, glycine, histidine, lysine, methionine, serine, threonine, and tryptophan) were significantly lower in the CAP group than in the control group on Day 0 (Figs. 1 and 2), but phenylalanine was considerably higher. Of the amino acids that decreased on Day 0, 3 amino acid levels (citrulline, histidine, and tryptophan) in males remained low on Day 7. However, asparagine, aspartic acid, ornithine, proline, and threonine were significantly higher on Day 7 in the CAP group than in the control group in both males and females (Figs. 1 and 2). On Day 7, most of the other amino acids were similar in CAP group and controls.
4. Discussion

In this study, differences were observed in the levels of 13 amino acids between CAP group and healthy controls in both male and female. As in previous studies [19], patients with CAP had higher phenylalanine levels than those of controls on Days 0 and 7. And the levels of six amino acids (asparagine, citrulline, glutamine, histidine, serine, and threonine), which were reduced in the CAP group, have been shown to be decreased in patients with acute exacerbations of chronic obstructive pulmonary disease with bacterial infection [6]. Cytokines and metabolites produced from the host during the bacterial infection are similar in some respects and different in others, between gram-positive and gram-negative bacteria [20,21]. However, we did not find any significant differences in amino acid levels according to Gram stain type. Therefore, we combined them for comparison with the control group. However, an *in vivo* study of bacterial infection demonstrated increases in some amino acids (e.g., alanine, histidine, leucine, tyrosine, valine) [22], in contrast to the findings of this study. Thus, there may be marked differences in the metabolic response to infection between humans and other animals.

Arginine, aspartic acid, citrulline, and ornithine are amino acids that are involved in the urea cycle [18]. The CAP group had lowered plasma levels of arginine and citrulline on Day 0, while the levels of aspartic acid and ornithine were not lowered. However, aspartic acid and ornithine were increased on Day 7. Arginine is a substrate for nitric oxide (NO) production and is known to be involved in inflammation. A previous report described the relationship between citrulline, arginine, and ornithine in the context of NO production [23]. During the bacterial infection, arginine and citrulline play important roles in disease pathogenesis [24], and arginine metabolism regulates host immunity [25]. These findings indicate that the metabolism of these amino acids in urea cycle and NO production significantly changed during the acute phase of bacterial pneumonia, modifying host immunity.

Alanine, cystine, glycine, serine, threonine, and tryptophan are metabolized to produce pyruvic acid (Table 4) [18]. Glycogenesis starts with the pyruvic acid produced by these amino acids [18]. These amino acids, except cystine, were decreased in the CAP group on Day 0. Cystine was the only amino acid that was not decreased in the CAP group on Day 0. Cystine is a substrate for nitric oxide (NO) production and is known to be involved in inflammation. A previous report described the relationship between citrulline, arginine, and ornithine in the context of NO production [23]. During the bacterial infection, arginine and citrulline play important roles in disease pathogenesis [24], and arginine metabolism regulates host immunity [25]. These findings indicate that the metabolism of these amino acids in urea cycle and NO production significantly changed during the acute phase of bacterial pneumonia, modifying host immunity.

4. Discussion

In this study, differences were observed in the levels of 13 amino acids between CAP group and healthy controls in both male and female. As in previous studies [19], patients with CAP had higher phenylalanine levels than those of controls on Days 0 and 7. And the levels of six amino acids (asparagine, citrulline, glutamine, histidine, serine, and threonine), which were reduced in the CAP group, have been shown to be decreased in patients with acute exacerbations of chronic obstructive pulmonary disease with bacterial infection [6]. Cytokines and metabolites produced from the host during the bacterial infection are similar in some respects and different in others, between gram-positive and gram-negative bacteria [20,21]. However, we did not find any significant differences in amino acid levels according to Gram stain type. Therefore, we combined them for comparison with the control group. However, an *in vivo* study of bacterial infection demonstrated increases in some amino acids (e.g., alanine, histidine, leucine, tyrosine, valine) [22], in contrast to the findings of this study. Thus, there may be marked differences in the metabolic response to infection between humans and other animals.

Arginine, aspartic acid, citrulline, and ornithine are amino acids that are involved in the urea cycle [18]. The CAP group had lowered plasma levels of arginine and citrulline on Day 0, while the levels of aspartic acid and ornithine were not lowered. However, aspartic acid and ornithine were increased on Day 7. Arginine is a substrate for nitric oxide (NO) production and is known to be involved in inflammation. A previous report described the relationship between citrulline, arginine, and ornithine in the context of NO production [23]. During the bacterial infection, arginine and citrulline play important roles in disease pathogenesis [24], and arginine metabolism regulates host immunity [25]. These findings indicate that the metabolism of these amino acids in urea cycle and NO production significantly changed during the acute phase of bacterial pneumonia, modifying host immunity.

Alanine, cystine, glycine, serine, threonine, and tryptophan are metabolized to produce pyruvic acid (Table 4) [18]. Glycogenesis starts with the pyruvic acid produced by these amino acids [18]. These amino acids, except cystine, were decreased in the CAP group on Day 0. Cystine was the only amino acid that was not decreased in the CAP group on Day 0. This suggests a selective use of amino acids for glycogenesis in bacterial pneumonia. The metabolism of glycine, histidine, and threonine affects the efficiency of complement-mediated bacterial killing [26]. Glycine has been proposed to be an anti-inflammatory nutrient [27] because it inhibits inflammatory cell activation [27] and reduces inflammatory cell influx into the lung tissue [28]. Therefore, it was considered that a reduction in glycine level may attenuate the inhibition of inflammatory cell activation, i.e., a reduction in glycine level in the early stage of bacterial pneumonia may enhance the inflammatory reaction.

Arginine, glutamine, histidine, proline, and ornithine are metabolized to glutamic acid [18]. Moreover, arginine and glutamine are considered important nutrients for immunity [4]. In this study, the levels of these amino acids, except for ornithine and...
Fig. 1. Intergroup comparisons for each amino acid in males of both groups. 

Notes: There were 50 control males, 36 males with CAP on day 0, and 24 males with CAP on day 7. The horizontal solid lines indicate the mean values in each group. Statistical analysis was performed using the Steel-Dwass method.

Abbreviations: CAP, community-acquired pneumonia; C, healthy male control (group C); Day 0, CAP male within 24 h after hospitalization; Day 7, CAP males 6–8 days after hospitalization.
Fig. 2. Intergroup comparisons for each amino acid in females of both groups

Notes: There were 43 control females, 24 females with CAP on Day 0, and 17 females with CAP on Day 7. The horizontal solid lines indicate the mean values in each group. Statistical analysis was performed using the Steel-Dwass method.

Abbreviations: CAP, community-acquired pneumonia; C, healthy female control (group C); Day 0, CAP female within 24 h after hospitalization; Day 7, CAP females 6–8 days after hospitalization.
proline, were decreased Day 0. The reduction in arginine level may be related to the metabolic cross talk between the host and the pathogen, which is discussed later. Glutamine is the most abundant free amino acid in the body and is the most important gluconeogenic amino acid [29]. Glutamine is also the primary fuel for immune cells in the catabolic setting and is an effective nutrient for immunity [30]. Thus, the reduction in glutamine level on Day 0 may have been due to consumption. l-Histidine augments the oxidative damage of gram-negative bacteria induced by hydrogen peroxide [31], and histidine is a precursor of histamine and contributes to inflammation in pneumonia [32]. The decrease in histidine level on Day 0 may also be associated with the metabolic cross talk between the host and the pathogen [9]. On the other hand, glutamic acid, ornithine, and proline levels were not decreased on Day 0. These results show that different changes occur in the plasma levels of amino acids that share metabolic pathways toward 2-oxoglutaric acid through glutamic acid [18].

In the present analysis, phenylalanine increased on Day 0 and Day 7 (Figs. 1 and 2). Plasma phenylalanine is known to increase with either bacterial or viral infection [19,33]. The explanation is that the catabolism of muscular skeletal protein related to infection results in phenylalanine release, and the released phenylalanine is used in the synthesis of inflammation-related substances in the liver. The reduction of amino acids on day 0 were resolved on day 7, except for three amino acids (citrulline, histidine, and tryptophan) in male group. Furthermore, the levels of five amino acids (marked with \* in Table 4) became higher than those of the control group on Day 7 in both male and female. All increased amino acids are glycogenic. Furthermore, these five amino acids are not metabolized to acetoacetic acid. This excessive recovery seems characteristic of the convalescent stage of bacterial pneumonia.

Each amino acid has been shown to be involved in inflammation and the immune response, leading to the concept of “immunonutrition” [4]. This study showed that individual amino acid level differed significantly in the early and recovery phases of CAP. There are amino acids, such as methionine, that are essential for bacterial proliferation [34]. And l-alanine and l-lysine are the main constituent elements of the peptidoglycans in the bacterial wall [35]. On the other hand, the decrease in asparagine arrests group A Streptococcus growth [36]. And tryptophan depletion by indoleamine 2,3-dioxygenase induction in infection is a recognized antimicrobial defense mechanism, which mediates immunoregulatory effects [37,38]. In bacterial infection, the total plasma amino acid decreases before the onset of clinical symptoms such as fever [19]. Therefore, it is considered that the reductions in these amino acids are not only due to enhanced metabolism related to fever or antibody production, but are also induced by the host-mediated response against bacterial infection. Furthermore, metabolic cross talk exists between the host and the pathogen. For example, the host and pathogen compete for specific amino acids such as arginine, asparagine, and tryptophan [5,39]. The host uses arginine as a substrate for NO production, while the pathogen activates arginase to deplete the host arginine levels. Further, asparagine is required for host T cell activation and differentiation, and is an important source of nitrogen for the pathogen. There remain many unclarified points in this area, and the interactions between individual amino acids and immunity in bacterial pneumonia are potential topics for future studies. For example, in recent years, the lung microbiome has been studied with regard to its role in bacterial pneumonia [40]. The interactions between the microbiome and the changes in plasma amino acids in patients with bacterial pneumonia should also be investigated in future studies.

There are some limitations to this study. Those in the CAP group were unable to fast since pneumonia treatment was a priority. Thus, the possibility that the infusion of electrolyte solution with glucose or lactic acid and the intake of lunch somewhat affected the results cannot be excluded. The metabolism of arginine, proline, and pyruvate has been extracted for the discrimination of severity [41]. However, this study did not include patients categorized as ‘very severe,’ and did not discuss the correlation between the severity of pneumonia and the changes in amino acids. The time course of plasma amino acids from the acute to convalescent phase in very severe pneumonia warrants further study. The topic of sex-related differences in amino acid changes, which was observed in this study, also needs to be studied further.

5. Conclusions

The levels of 12 amino acids were significantly lower in CAP group than in healthy controls at the acute phase of bacterial pneumonia, and this is likely to have been attributable to the bacterial infection. During convalescence, the levels of some amino acids differed from those on admission and those of healthy controls, suggesting that there is a distinct amino acid metabolism profile during the convalescent phase following bacterial infection. Based on these findings, reconsideration for providing amino acids to patients with bacterial pneumonia should be needed depending on stage of bacterial pneumonia from the perspective of immunonutrition.

Statement of authorship

HI had full access to the study data, takes full responsibility for the integrity of the data and the accuracy of the analysis, contributed to the study design, contributed to writing of the manuscript, the data analysis, drafting and revising the paper, and agree to be accountable for all aspects of the work.
Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest
The author reports no conflicts of interest in this work.

Acknowledgments
I would like to thank Kanako Yoshida for treatment of the blood samples. I would like to thank Editage (https://www.editage.jp) for English language editing and SRL, Inc (http://www.srl-group.co.jp/english/index.html) for analyzing the amino acids.

References