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Serum Amyloid: A as an Indicator of Infection in Donkeys

Junqiao Li[1], Guimiao Jiang[2], Juntao Zhang[1], Weichao Liang[1], Fuwei Zhao[2], Weiping Gao[2], Shaojun Liu[1], Chao Wang[2], Lixin Deng[1] and Zhiping Zhang[1*]

[1]Department of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou, China

[2]National Engineering Research Center for Gelatin-Based Traditional Chinese Medicine, Shandong Province, China

Abstract

Acute Phase Proteins (APPs) are those produced by the liver following stimulation by stress, such as trauma, infection or inflammation. Changes in their concentration in blood have been used as important indicators of disease, and widely used in the diagnosis of diseases in humans and pets, such as cats and dogs. Donkeys and horses have a clinical response to disease that is distinct, in many cases, such as having more tolerance to pain and displaying fewer obvious clinical symptoms. The principal APP varies by species and remains unknown for donkeys. 33 donkeys with the clinical disease (8 young, 25 adult) and 37 clinically healthy donkeys (10 young, 27 adult) were included in the current study, for evaluating for clinically important APPs in donkeys. Blood was collected from the jugular vein of all donkeys and a Complete Blood Count (CBC) and standard blood biochemistry report conducted. Serum Amyloid A (SAA), Haptoglobin (HP), C-Reactive Protein (CRP) and α1-Acidic Glycol Protein (α1-AGP) in the blood samples were quantified by enzyme linked immunosorbent assay. The results indicated that SAA and α1-AGP in the young, clinically-ill donkeys were significantly higher in concentration than in young healthy donkeys ($P<0.05$). The difference in SAA ($P<0.01$) and HP concentrations ($P<0.05$) were also significantly higher in adult clinically-ill donkeys than in adult healthy donkeys. Conversely, there was no difference in CRP concentrations in healthy and clinically ill donkeys ($P>0.05$). The young clinically ill donkeys had significantly higher numbers of Red Blood Cells (RBCs) and had a Higher Hematocrit (HCT) and higher levels of Hemoglobin (HG) than young healthy donkeys ($P<0.01$), as were levels of Aspartate Transaminase (AST), urea and Phosphate ($P<0.05$). The adult clinically ill donkeys had significantly higher numbers of White Blood Cells (WBC), neutrophils and monocytes than adult healthy donkeys ($P<0.01$), while conversely, numbers of eosinophils ($P<0.01$) and platelets ($P<0.05$), hematocrit ($P<0.01$) and levels of hemoglobin ($P<0.01$) were higher in the adult healthy donkeys. Total Protein (TP), urea, creatinine and glucose concentrations were higher in adult clinically ill donkeys than in adult healthy donkeys ($P<0.05$). Taken together, the results of this study indicate that a significant difference in SAA blood concentration was observed in healthy donkeys compared to those that were clinically ill and thus is responsive to the health of donkeys. Therefore, SAA can be considered the principal APP of donkeys for the early diagnosis of clinical disease in donkeys.

Keywords

Donkeys • Acute phase protein • Serum amyloid A • Haptoglobin • C-reactive protein • α1-acidic glycoprotein

Introduction

Macrophages and neutrophils secrete cytokines such as TNF-α, IL-1 and IL-6 when stimulated by stress such as trauma, infection or inflammation. These cytokines combine with specific receptors in the liver to induce hepatocyte synthesis and the secretion of proteins into the blood. Changes in concentration may directly reflect the degree of infection, and the proteins whose levels change are collectively referred to as Acute Phase Proteins (APPs), including Serum Amyloid A (SAA), Haptoglobin (HP), C-Reactive Protein (CRP) and α1-Acid Glycol Protein (α1-AGP). APPs are categorized as either positive or negative, with positive APPs being divided into proteins that are main, medium or secondary [1,2]. Studies have demonstrated that APPs may be useful as quantitative biomarkers for assessing the systemic response of the innate immune system to infection, inflammation or trauma, and potentially useful for prognosis and in monitoring the response to treatment [3,4]. As indicators of inflammation, APPs have been widely used in clinical practice, and the acute phase proteins such as SAA, HP, CRP, α1-AGP can be used to monitor the health and condition of animals, and so is an effective means to estimate the incidence of infectious diseases in livestock [5]. Using APPs in veterinary clinical practice has been considered an economic and effective method. SAA is the main APP in horses, with its serum concentration rapidly increasing more than 100 folds after trauma, infection or inflammation. Researchers have previously evaluated the utility of SAA in healthy horses and a newborn foal sepsis [6]. CRP is the main APP in dogs, its rise in concentration being similar to that of SAA in horses in response to diseases such as Babesiosis, Leishmaniasis, leptospirosis, arthritis, blood diseases and lymphoma. With regard to cats, SAA rapidly responds to a variety of inflammatory stimuli and is thus the most effective APP to measure [4]. Studies have shown that although donkeys and horses are similar, they react differently too many clinical illnesses, such as their reaction to pain during angina attacks and numerous infectious diseases. Donkeys are generally more tolerant and so early clinical symptoms are difficult to detect. Therefore, the reliability of diagnosing donkeys by reference to common symptoms of horses remains to be studied [7]. The condition of horses is normally judged by detection of APPs in the blood, but no reports of APPs in donkeys have been published, so the aim of this study was to screen the main APP in donkeys to provide a reference to diagnose clinical illness.

***Address for Correspondence:** Zhiping Zhang, Department of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou, China; Tel: 13603983584; Email: zhipingzhang@henau.edu.cn*

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Materials and Methods

Experimental animals and sample collection

In 2017, 10 young healthy donkeys (6 months old, mean mass approximately 121 kg), 8 young clinically ill donkeys (6 months old, mean mass approximately 115 kg), 27 adult healthy donkeys (24 months old, mean mass approximately 203 kg) and 25 adult clinically ill donkeys (24 months old, mean mass approximately 208 kg) were selected from the national black donkey breeding base in Dong'e county, Liaocheng city, Shandong province in China. Healthy donkeys were housed in fattening pens, while clinically ill donkeys were housed in isolation.

Blood sample collection was performed by jugular venipuncture. 2 ml of donkey blood were collected into a tube containing K2-EDTA which was gently

shaken and used for a complete blood count (CBC, Mind ray BC-5000). A further 5 ml of blood were collected for blood biochemistry (Mind ray BS-180), using a common vacuum blood collection tube. The blood was centrifuged at 3400 × g for 10 min and the supernatant stored at -20°C until required for the APP concentration test. An APP test kit for horses can be purchased but no such test exists for donkeys. A comparison of the amino acid sequences of CRP, HP and SAA in horses and donkeys found >97% homology. Therefore, the same kits were used for the quantification of concentration of APPs in donkey blood, namely horse SAA Enzyme-Linked Immune Sorbent Assay (ELISA), horse CRP ELISA, horse HP ELISA and horse α1-AGP ELISA kits, provided by Shanghai Enzyme-Linked Biotechnology Co., Ltd.

Experimental design

10 young healthy donkeys, 8 young clinically ill donkeys (with joint swelling and diarrhea), 27 adult healthy donkeys and 25 adult clinically ill donkeys (with diarrhea and difficulty in abdominal breathing,) were selected for the study. Blood was sampled from the jugular vein of each and a CBC and blood biochemistry conducted and the concentrations of SAA, HP, CRP and α1-AGP quantified.

Data analysis

Experimental results are expressed as mean ± standard deviation. Statistical analysis was conducted using SPSS version 19 software. Significant differences were established by analysis with independent sample T tests. P<0.05 was used as the threshold for significance, expressed by *, while P<0.01 was considered extremely significant, expressed by **.

Results

Complete blood count

The results in Table 1 demonstrated that the Red Blood Cell (RBC), Hemoglobin (HGB) and Hematocrit (HCT) values in the young clinically ill donkeys were significantly higher than in young healthy donkeys (P<0.01). The numbers of White Blood Cells (WBC), Neutrophils (Neu) and Monocytes (Mon) in adult clinically ill donkeys were significantly higher than in adult healthy donkeys (P<0.01). However, Eosinophil (Eos) numbers (P<0.01), levels of HGB (P<0.01), HCT (P<0.01) and platelet (PLT) (P<0.05) numbers in adult healthy donkeys were higher than in adult clinically ill donkeys.

Table 1. Blood routine index test results of healthy young donkeys and apparent sick young donkeys.

Routine blood cell indexes	Healthy young donkeys (n=10)	Apparent sick young donkeys (n=8)
WBC (10^9/L)	14.04 ± 1.93	14.31 ± 3.2
Neu ($10^{\wedge 9}$/L)	5.47 ± 1.33	4.73 ± 0.89
Lym (10^9/L)	7.41 ± 1.51	6.31 ± 1.63
Mon (10^9/L)	0.51 ± 0.08	0.48 ± 0.17
Eos (10^9/L)	0.45 ± 0.13	0.52 ± 0.2
Bas (10^9/L)	0.11 ± 0.06	0.12 ± 0.05
RBC ($10^{\wedge 12}$/L)	6.89 ± 0.51	9.06 ± 0.48
HG (g/L)	121.4 ± 7.27	142.56 ± 32.59
HCT (%)	39.58 ± 2.55	48.43 ± 6.03
PLT (10^9/L)	189.35 ± 52.85	191.79 ± 66.63
PCT (%)	0.13 ± 0.03	0.11 ± 0.04

Blood biochemistry panel

The results in Table 2 demonstrate that Aspartate Transaminase (AST), urea and phosphate concentrations in the blood from young clinically ill donkeys were higher than in young healthy donkeys (P<0.05), while Total Protein (TP), urea, Creatinine (CREA) and Glucose (GLU) concentrations in adult clinically ill donkeys were significantly higher than in adult healthy donkeys (P<0.01). The CBC and blood biochemistry data demonstrated significant differences between the clinically ill and healthy donkeys.

Table 2. Blood biochemical indexes of healthy young donkeys and apparent sick young donkeys.

Routine blood biochemical indexes	Healthy young donkeys (n=10)	Apparent sick young donkeys (n=8)
AST(U/L)	229.3 ± 12.75	266.66 ± 41.12
TP (g/L)	55.21 ± 3.28	52.09 ± 2.79
ALB (g/L)	26.08 ± 1.44	27.01 ± 1.3
UREA (mmol/L)	3.12 ± 1.21	6.87 ± 0.96
CREA (μmol/L)	104.98 ± 10.08	112.79 ± 6.83
GLU (mmol/L)	6.66 ± 0.89	6.80 ± 1.05
CK (U/L)	151.69 ± 24.6	217.91 ± 42.47
Ca (mmol/L)	3.23 ± 0.08	3.25 ± 0.11
P (mmol/L)	1.62 ± 0.07	1.85 ± 0.11
TG(mmol/L)	0.38 ± 0.05	0.42 ± 0.16
TC(mmol/L)	1.69 ± 0.12	2.13 ± 0.29
LDH(U/L)	262.10 ± 56.09	253.99 ± 30.54

Concentration of acute phase proteins in blood

As shown in Figure 1 the concentration of CRP in the blood of the young donkeys was significantly higher than that of the adult donkeys (P<0.01) within the same health category. However, for each age category, there was no difference in CRP value between the healthy donkeys and clinically ill donkeys.

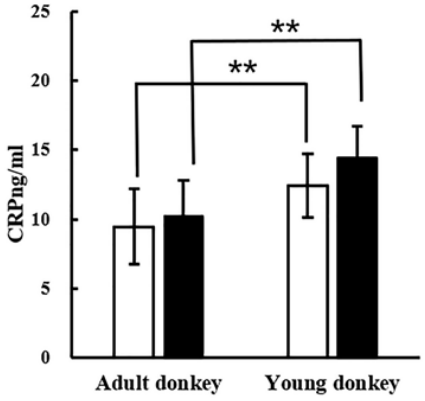

Figure 1. CT scan with contrast showing inflammation in wall of aorta and Morita class III coeliac trunk anomaly. **Note:** (□) Healthy donkey; (■) Clinically ill donkey

As shown in Figure 2 the concentration of HP in the blood of the adult clinically ill donkeys was significantly higher than in the adult healthy donkeys (P<0.01). In the young clinically ill donkeys the concentration was higher than in the young healthy donkeys, but not significantly so (P>0.05). Within the same health category, there was no difference between young and adult donkeys (P>0.05).

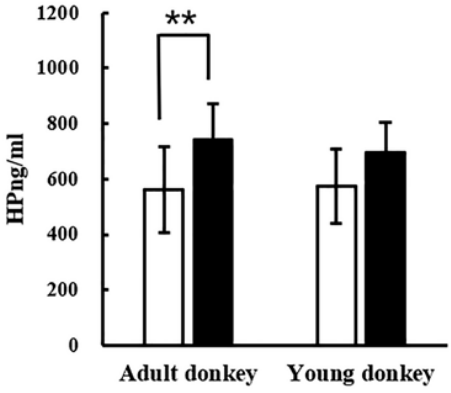

Figure 2. Concentration of HP in donkeys.**Note:** (□) Healthy donkey; (■) Clinically ill donkey

As shown in Figure 3 the concentration α1-AGP in the blood of the young clinically ill donkeys was higher than that of the young healthy donkeys (P<0.05). The concentration adult healthy donkeys were higher than adult clinically ill donkeys but not significantly so (P>0.05). Within the same health category, there was no difference between young and adult donkeys (P>0.05).

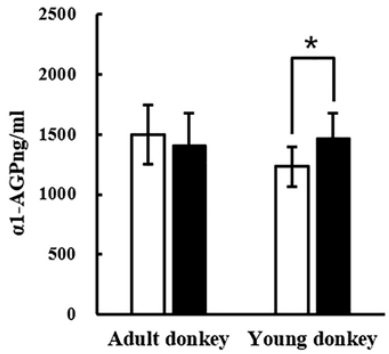

Figure 3. Concentration of α1-AGP in donkeys. **Note:** (□) Healthy donkey; (■) Clinically ill donkey

As shown in Figure 4 the concentration of SAA in the blood of adult clinically ill donkeys was significantly higher than that of the adult healthy donkeys (P<0.01) and the concentration in young clinically ill donkeys was higher than in young healthy donkeys (P<0.05). Within the same health category, there was no difference between young and adult donkeys (P>0.05).

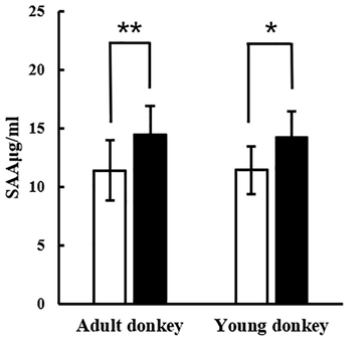

Figure 4. Concentration of SAA in donkeys. **Note:** (□) Healthy donkey; (■) Clinically ill donkey

Discussion

Complete blood counts and blood biochemistry panels are the most commonly used tests in clinical practice. They can be used to judge the health of a presenting animal and allows veterinarians to better understand the occurrence and development of a disease. Studies have shown that in many diseases (such as chronic kidney disease, rheumatoid arthritis, etc.) the Neu to Lym ratio or PLT to Lym ratio are satisfactory indicators for the evaluation of disease [8-11]. Lippi et al. evaluated a large cohort of samples and demonstrated that the width of the RBC distribution (RDW) increased significantly during an inflammatory response, which can be used as a diagnostic indicator of the degree of inflammation. In addition, RDW is also a parameter of erythrocyte volume heterogeneity, and a diagnosis ofanemia would also manifest the same result [12]. The blood indices commonly used in clinical practice to reflect liver injury are ALT, AST, GGT, etc. Takeuchi et al. [13] found that increased levels of cystatin C were also associated with chronic liver disease. In the present study, RBC, HGB and HCT values in the blood of young clinically ill donkeys were significantly higher than those of young healthy donkeys (P<0.01), with AST, urea and phosphate concentrations in the blood of young clinically ill donkeys higher than in young healthy donkeys (P<0.05). WBC, Neu and Mon values in the blood of the adult clinically ill donkeys were significantly higher than those of the adult healthy donkeys (P<0.01). However, Eos (P<0.01), HGB (P<0.01), HCT (P<0.01) and PLT values in adult healthy donkeys were higher than in adult clinically ill donkeys. The clinical symptoms of the young clinically ill donkeys were mostly diarrhea and rhinorrhea in the adult clinically ill donkeys. Apparent elevation of RBC numbers may be due to dehydration caused by diarrhea and thus increased RBC concentration. Similarly, elevation of urea may also be due to fluid loss for the same reason, a concomitant reduction in renal blood flow and a reduction in glomerular filtration rate. WBC numbers should be elevated following an inflammatory response. WBC numbers in adult clinically ill donkeys were higher than in adult healthy donkeys, suggesting the presence of inflammation. However, the numbers of WBCs in young clinically ill donkeys was no different than those observed in healthy donkeys. It may be that some clinically ill donkeys had adapted to the conditions of stress or had returned to a normal condition from the stress conditions.

In recent years, a number of studies have confirmed that APPs can be used as a high sensitivity index for the diagnosis of inflammation, but lacking specificity and principally utilized in animal species [4]. Previous studies have found that the concentration of CRP in dogs does not change with age, being significantly associated with the scope of inflammation and its severity [14]. Thus, it is used as an indicator of canine cystitis and uterine abscess, and can also be used for monitoring treatment processes and evaluating disease prognosis [15,16]. In the present study, the concentration of CRP in the blood of young donkeys was significantly higher than that of adults (P<0.01) within the same health category. This demonstrates that CRP concentration in donkeys is related to the age of donkeys. For a particular age, no difference was observed in the healthy donkeys compared to the clinically ill donkeys (P>0.05), indicating that CRP concentration in donkey blood is not related to its health. However, this is not consistent with existing reports. It may be that CRP is not responsive in donkeys and so cannot be used as their main APP.

The concentration of HP and α1-AGP in animals of all ages is widely used as an auxiliary index for disease diagnosis. Skinner established that HP can be used as an effective indicator of bacterial infection in sheep which is more sensitive and specific than other hematological indicators. It is widely used to monitor whether uterine inflammation is present in ewes [5,17]. Studies have shown that the acute phase protein, α1-AGP, has been detected in pigs with pneumonia and meningitis [18]. In dogs with tumors, increased concentrations of α1-AGP is strongly positively correlated with the presence of sialic acid, which is of diagnostic significance in oncogenic diseases [19]. In the present study, the concentration of HP in adult clinically ill donkeys was significantly higher than that of the adult healthy donkeys (P<0.01), a result not observed in young donkeys. In addition, there was no difference in HP concentration between young and old donkeys within the same health category. The concentration of α1-AGP in young clinically ill donkeys was higher than in young healthy donkeys (P<0.05), but not in adult donkeys, or between adult and young donkeys within the same health category. Therefore, HP and α1-AGP have limitations so far as their utility in the monitoring of clinical disease in donkeys of different ages, and so cannot be utilized as their main APP.

SAA is the main APP in cows and horses and can sensitively reflect bovine mastitis, bovine viral diarrhea and horse respiratory diseases, especially influenza and malarial pneumonia [20].

Conclusion

In the present study, the concentration of SAA in adult clinically ill donkeys was significantly higher than in adult healthy donkeys (P<0.01), and similarly with young donkeys (P<0.05). Within the same health category, no difference was observed between the young and adult donkeys (P>0.05). In conclusion, compared with the other three APPs, SAA concentration is not affected by age in donkeys and has higher sensitivity. Therefore,

SAA can be used as the main APP to monitor the health of donkeys. The concentration of SAA in the blood of donkeys will provide an important reference for the diagnosis of disease in donkeys.

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Conflict of Interest

The authors of this article do not have a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the article.

References

1. Petersen, HH, Nielsen JP and Heegaard PMH. "Application of Acute Phase Protein Measurement in Veterinary Clinical Chemistry." *Vet Res* 35(2004):163-187.
2. Ebersole, Jeffrey L and Cappelli David. "Acute Phase Reactants in Infections and Inflammatory Diseases." *J Periodontol* 23(2000): 19-49.
3. Ceron, Jose Joaquin, Peter David Eckersall and Silvia Martínez Subiela. "Acute Phase Proteins in Dogs and Cats: Current Knowledge and Future Perspectives." *Vet Clin Pathol* 34(2005): 85-99.
4. Eckersall, PD and R Bell. "Acute Phase Proteins: Biomarkers of Infection and Inflammation in Veterinary Medicine." *J Vet* 185(2010): 23-27.
5. Skinner, JG and L Roberts. "Haptoglobin as an Indicator of Infection in Sheep." *Vet Rec Open* 134(1994): 33-36.
6. Cohen, Noah D, MK Chaffin, ML Vandenplas and RF Edwards, et al. "Study of Serum Amyloid Concentrations as a Means of achieving Early Diagnosis of Rhodococcus Equi Pneumonia." *J Equine Vet* 37(2005):212-216.
7. Laus, F, E Paggi, M Cerquetella and D Spaziante, et al. "Guttural Pouch Mycosis in a Donkey (Equus Asinus): A Case Report." *Vet Med* 561(2010).
8. Boyraz, Ismail, Bunyamin Koc, Ahmet Boyac and Ahmet Tutoglu, et al. "Ratio of Neutrophil/Lymphocyte and Platelet/Lymphocyte in Patient with Ankylosing Spondylitis that are Treating with Anti-TNF." *Int J Clin Exp Med* 9(2014):2912.
9. Kim, Hyoun Ah, Ju Yang Jung, and Chang-Hee Suh. "Usefulness of Neutrophil-to-Lymphocyte Ratio as a Biomarker for Diagnosing Infections in Patients with Systemic lupus Erythematous." *Clin Rheumatol* 36(2017): 2479-2485.
10. Kucuk, A, AU Uslu, Y Ugan and S Bagcaci, et al. "Neutrophil-to-Lymphocyte Ratio is Involved in the Severity of Ankylosing Spondylitis." *Bratisl Lek Listy* 116(2015): 722-725.
11. Lippi, Giuseppe, Giovanni Targher, Martina Montagnana and Gian Luca Salvagno, et al. "Relation between Red Blood Cell Distribution Width and Inflammatory Biomarkers in a Large Cohort of Unselected Outpatients." *Arch Path Lab* 133 (2009): 628-632.
12. Van Zeben, Derkjen, Robert Bieger, Robert KA Van Wermeskerken and Ad Castel, et al. "Evaluation of Microcytosis Using Serum Ferritin and Red Blood Cell Distribution Width." *Eur* 44(1990): 106-109.
13. Takeuchi, Mamiko, Yoshihide Fukuda, Isao Nakano and Yoshiaki Katano, et al. "Elevation of Serum Cystatin Concentrations in Patients with Chronic Liver Disease." *Eur J Gastroenterol Hepatol* 13(2001): 951-955.
14. Eckersall, PD. "Recent Advances and Future Prospects for the Use of Acute Phase Proteins as Markers of Disease in Animals." *Rev Med Vet* 151(2000):577-584.
15. Seo, Kyoung Won, Jong bok Lee, Jin-Ok Ahn and Hee-woo Lee, et al. "Creactive Protein as an Indicator of Inflammatory Responses to Experimentally Induced Cystitis in Dogs." *J Vet Sci* 13(2012):179-185.
16. Jitpean, Supranee, Bodil Ström Holst, Odd V Hoglund and Ann Pettersson, et al. "Serum Insulin Like Growth Factor-I, Iron, Creactive Protein, and Serum Amyloid A for Prediction of Outcome in Dogs with Pyometra." *Theriogenology* 82(2014):43-48.
17. Sobiesiak, M. "Acute Phase Proteins in Transplant Patients." *Transplantation* 15(2003): 25-30.
18. Koruk, Mehmet, S Taysi, M C Savas and Omer Yilmaz,et al. "Serum Levels of Acute Phase Proteins in Patients with Nonalcoholic Steatohepatitis." *Urk J Gastroenterol* 14(2003):12-17.
19. Sonne, C, S Fonfara, R Dietz and M. Kirkegaard,et al. "Multiple Cytokine and Acute-Phase Protein Gene Transcription in West Greenland Sledge Dogs (Canis Familiaris) Dietary Exposed to Organic Environmental Pollutants." *Arch Environ Contam Toxicol* 53(2007):110-118.
20. Jacobsen, S, and P H Andersen. "The Acute Phase Protein Serum Amyloid (SAA) as a Marker of Inflammation in Horses." *Equine Vet Educ* 19(2007):38-46.

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