

Original Article

Comparison of the Effect of Amino Acids and Their Derivatives on the Growth of Some Dermatophytes: An *In Vitro* Study

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ABSTRACT

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Key words

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Background and Aims: Amino acids have different effects on the growth of some dermatophytes. Some may increase whereas others may decrease their growth. The concentration of some amino acids is also an important factor for their effects.

Materials and Methods: To investigate the effects of L-amino acids on the growth of six species of dermatophytes including *Epidermophyton floccosum*, *Microsporium gypseum*, *Microsporium canison*, *Trichophyton rubrum*, *Trichophyton schoenleinii* and *Trichophyton verrucosum*, two concentrations (1 and 0.1 mg/ml) of the 23 L-amino acids and some of their derivatives were added to sabouraud glucose agar media of these dermatophytes. The experiment was carried out three times. After 3 weeks for *Trichophyton verrucosum* and *Trichophyton schoenleinii*, and 2 weeks for the rest of dermatophytes, the mean diameter of each colony was measured and compared with the control whose media was not treated with amino acids.

Results: The results showed the higher inhibitory effects of L-Cysteine hydrochloride, L-Cysteine, L-Aspartic acid, L-Glutamic acid, DL-Tryptophan and L-Tyrosine on the studied dermatophytes. The other amino acids had less inhibitory or even stimulatory effects on the growth of the dermatophytes.

Conclusions: By using the properties of these effective amino acids, antifungal drugs may be synthesized more effective with lower cost and less side effects against different dermatophytes.

Introduction

Dermatophytes belong to a group of organisms that are able to break down the keratin in tissues such as the epidermis, hair, nails, feathers, horns and hooves. A common contagious disease caused by dermatophytes is named Dermatophytosis [1, 2]. Some dermatophytes (anthropophilic species) are adapted to humans and are usually transmitted from person to person. Others (zoophilic species) are adapted to animals or soil saprophyte (geophilic), capable of invading man and animal tissue.

Dermatophyte species are distributed in three generic shapeless forms, taxonomically correlated as follows: *Trichophyton*, *Microsporum* and *Epidermophyton* all of which belong teleomorphously to a unique gender- Arthroderma [3, 4]. They show variety in space and time, i.e, some are geographic and universally distributed as the *Trichophyton rubrum* (*T. rubrum*) and others are limited to a continent or region as *Trichophyton concentricum* fungus [4]. In addition, depending on their primary adaption to animals, soil or humans, species of dermatophytes are divided into zoophilic, geophilic or anthropophilic, respectively [5, 6]. Zoophilic species are responsible for about 30% of human dermatophytoses and often provoke acute inflammation; anthropophilic species represent about 70% of infections on these hosts, causing a chronic infection of slow progression, suggesting that the fungus adapts to the human host. Of human pathogens, so far about 30 species of dermatophytes have been identified [7]. Some factors including climatic factors, social practices, migration and

individual characteristics may influence the epidemiology of dermatophytoses. Moreover, some factors such as age, genetic and illness may make some people sensitive and some resistant to dermatophytes [7]. Dermatophytes indicate deferent sensitivity toward effective factors and may also prefer specific areas of skin [8]; *Epidermophyton floccosum* (*E. floccosum*) prefers the groin area [9] and *Trichophyton mentagrophytis* (*T. mentagrophytis*) prefers palmar and plantar tissues [10].

Other factors such as damp, temperature and pH considered as physical factors [11], and steroids and fatty acids [12], blood group antigens [13], hormones and their metabolites [14, 15] as chemical agents may interfere with pathogenesis of dermatophytosis. Amino acid changes may be a risk factor for infection with dermatophytes in mammals [16]. Since keratin is composed primarily of amino acids, these components must influence, to a large extent, the ability of a dermatophyte to survive on the skin. Previous studies revealed that various amino acids might have inhibitory or stimulatory effects on the growth of some dermatophytes [17-19]. Certain amino acids are completely unavailable as initial source of nitrogen for a given species. Others are available, or at least beneficial, for growth while still others are inhibitory [20]. Therefore, many of the amino acids in keratin antagonize each other so that the inhibition or stimulation by one of them may be completely masked by its metabolic antagonist. In the present study, we investigated the effects of 23

amino acids with different concentrations on the growth of 6 dermatophytes including *E. floccosum*, *T. rubrum*, *Trichophyton schoenleinii* (*T. schoenleinii*), *T. verrucosum*, *Microsporum gypseum* (*M. gypseum*) and *Microsporum canison* (*M. canison*).

Material and Methods

Chemicals

The standard strains including *M. gypseum* (pTcc5070), *T. rubrum* (pTcc5143), *T. schoenleinii* (pTcc5221), *M. canison* (5069), and *T. verrucosum* (pTcc5056) were purchased from Fungal and Bacterial Collection of Iranian Scientific and Industrial Research Institute. Moreover, three strains of *E. floccosum* were isolated from lesional human skin and identified by standard criteria. L- Amino acids including L-Arginine, LD-Arginine, L-Histidine, L- Tyrosine, L- Cysteine hydrochloride, L- Cysteine, L-Methionine, L- Glycine, L- Asparagine monohydrochloride, L- Phenylalanine, L- Proline, L- Hydroxyproline, L- Histidine monohydrochloride, L- Threonine, Lysine monohydrochloride, L- Leucine, L- Isoleucine, L- Alanine, L- Valine, L- Serine, L- Glutamine, L- Aspartic acid and L-Glutamic acid were purchased from Merck.

Methods

Autoclaved sabouraud glucose agar (Merck) was supplemented with chloramphenicol (50 mg/l) and cyclohexamide (500 mg/l). Each amino acid was added to the cooled-down agar before pouring into the plates. Final concentrations of amino acids in the test plates were 0.1 and 1 mg/ml. The positive growth plates at 0.1 mg/ml amino acid concentration and negative at 1 mg/ml level were cultured

again on sabouraud glucose agar with amino acid concentrations of 0.25, 0.5 and 0.75 mg/ml to obtain the cutoff concentration. For each strain a control media of sabouraud glucose agar was considered without adding amino acid as control. Punch 4 mm in diameter was taken from the margins of fresh subculture growth on sabouraud glucose agar and used for standardized inoculation of all tests plates [14]. The experiment was carried out three times. Colony diameters were measured after 21 days for *T. verrucosum* and *T. schoenleinii*, and 14 days for the rest of dermatophytes at 25°C in the dark. Arithmetic means of thallus diameter were compared with students t-test by considering $p < 0.01$ as statistically significant.

Results

The different strains belonging to an identical specimen responded similarly to various amino acids. Therefore it was possible to evaluate the collective evolution per specimen. At first, the effects of two concentrations of each amino acids, 0.1 mg/ml and 1.0 mg/ml, on the growth of six dermatophytes were investigated by treating their media. The amino acids with the complete suppression effects on at least one dermatophyte at 1.0 mg/ml level, were selected and applied again in three concentrations below 1.0 mg/ml (0.25, 0.5 and 0.75 mg/ml) to determine the cutoff concentration and also to compare their effects on various dermatophytes. Among 23 amino acids, L- Cysteine hydrochloride, L- Cysteine, L- Aspartic acid, L- Glutamic acid, DL- Tryptophan and L- Tyrosine showed such property as shown in table 1. Also, table 1

shows the optimal concentration of each amino acid for the growth of each dermatophyte.

Table1. Comparison of the mean colony diameters of the dermatophytes in different concentrations of amino acids with strong inhibitory effects

Species	Amino acid	Concentration of amino acids (mg/ml)					
		0 (control)	0.1	0.25	0.50	0.75	1.0
<i>T. verrcosum</i>	L-Cys hydrochlorid	16.33 ±3*	43.00±1	0	0	0	0
	L-Cystine	16.33 ±3	21.33±0.6	12.33±1.5	5.33±2.1	0	0
	L-Aspartic acid	16.33 ±3	18.66 ±1.5	18.00±2.1	16.66±3.6	14.33±1.2	7.00±2.7
	L- Glutamic acid	16.33 ±3	18.00±2	18.00±1	17.67±2.1	11.00±1.7	0
	DL-Triptophan	16.33 ±3	17.00±3	16.67±1.5	16.33±2.5	16.33±1.5	16.33±1.7
	L-Tyrosine	16.33 ±3	17.67±1.2	17.33±1.2	16.3±2.1	16.4±1.5	10.67±2.6
<i>T. schoienlinii</i>	L-Cys hydrochlorid	22.33±1.2	24.67±0.6	0	0	0	0
	L-Cystine	22.33±1.2	26.67±0.6	24.33±1.2	22.00±1	21.67±1.2	0
	L-Aspartic acid	22.33±1.2	24.33±0.6	12.33±1.5	0	0	0
	L- Glutamic acid	22.33±1.2	22.33±1.2	16.00±1	11.33±1.2	10.33±1.5	3.67±(3.2)
	DL-Triptophan	22.33±1.2	21.33±1.2	20.00±1	19.33±0.6	16.33±0.6	0
	L-Tyrosine	22.33±1.2	23.67±0.6	22.33±0.6	21.67±0.6	17.67±1.2	0
<i>T. rubrum</i>	L-Cys hydrochlorid	39.67±4.93	43.34±1.5	0	0	0	0
	L-Cystine	39.67±4.93	45.00±1.7	42.67±1.5	31.67±2.5	27.67±1.2	0
	L-Aspartic acid	39.67±4.93	42.33±2.1	39.67±2.1	21.00±3.6	19±1.00	0
	L- Glutamic acid	39.67±4.93	43.00±1	41.00±1.7	40.00±1.7	32.67±1.5	0
	DL-Triptophan	39.67±4.93	42.00±1.7	48.00±2.7	44.33±1.2	34.67±2.1	0
	L-Tyrosine	39.67±4.93	43.00±1	43.00±1.7	41.33±1.5	36.67±2.5	20.33±2.5
<i>M. canison</i>	L-Cys hydrochlorid	47.00±1.7	48.33±1.2	0	0	0	0
	L-Cystine	47.00±1.7	46.67±1.5	38.67±2.5	36.33±3.1	27.33±2.5	0
	L-Aspartic acid	47.00±1.7	47.00±1	45.33±2.5	39.67±2.5	33.33±2.9	0
	L- Glutamic acid	47.00±1.7	38.67±1.2	36.00±1	32.33±1.5	29.67±2.5	25.33±1.5
	DL-Triptophan	47.00±1.7	49.33±2.5	36.00±3.1	33.67±2	27.00± 1.7	0
	L-Tyrosine	47.00±1.7	48.00±1.7	44.67±3.1	38.33±1.1	35.67±2.5	0
<i>M. gypsumand</i>	L-Cys hydrochlorid	52.33±1.5	45.33±3.58	0	0	0	0
	L-Cystine	52.33±1.5	52.00±2	52.00±3.1	51.33±2.1	46.67±3.1	0
	L-Aspartic acid	52.33±1.5	53.00±2	55.00±4.4	41.33±7.8	0	0
	L- Glutamic acid	52.33±1.5	52.33±2.1	47.00±2	42.67±1.2	13.67±1.5	0
	DL-Triptophan	52.33±1.5	48.67±1.2	49.33±0.6	43.33±1.2	37±1	0
	L-Tyrosine	52.33±1.5	59.00±1.5	48.33±3.1	47.67±1.7	47.33±3.1	45.00±2.1
<i>E. floccosum</i>	L-Cys hydrochlorid	16.67 ±0.58	15.00±1	0	0	0	0
	L-Cystine	16.67 ±0.58	18.00±1	14.33±0.6	10.67±1.2	8.33±3.5	0
	L-Aspartic acid	16.67 ±0.58	15.67±1.2	15.33±1.5	0	0	0
	L- Glutamic acid	16.67 ±0.58	16.00±1	15.00±2	10.33±1.5	0	0
	DL-Triptophan	16.67 ±0.58	16.67±0.6	15.67±1.2	10.67±1.2	6.33±1.5	0
	L-Tyrosine	16.67 ±0.58	17.00±0.6	15.67±1	14.67±1.2	13.33±0.6	4.00±3.6

*The mean colony diameter±SD (mm)

L- Cysteine hydrochloride appeared as the strongest growth inhibitor toward all six dermatophytes. The radial growth of all six dermatophytes were completely suppressed at 0.25 mg/ml of L- Cysteine hydrochloride while L- Cysteine completely suppressed their growth at 1 mg/ml concentration, except with *T. verrcosum* that was more sensitive and inhibited at 0.75 mg/ml concentration of L- Cysteine. Suppression property was followed by L- Aspartic acid with different sensitivities. Among six dermatophytes, *T. schoienlinii* and *E. floccosum* were the most sensitive to L- Aspartic acid at concentration of 0.5 mg/ml. The sensitivity to L- Aspartic acid levels decreased in *M. gypseum* at 0.75 mg/ml and *T. rubrum* and *M. canison* at 1.0 mg/ml. *T. verrcosum* was the most resistant dermatophyte to L-Aspartic acid.

E. floccosum was the most sensitive dermatophyte to L-Glutamic acid at cutoff concentration of 0.25 mg/ml. At cutoff concentration of 0.75 mg/ml, the sensitivity to L- Glutamic acid increased in *T. verrcosum* and *M. gypseum* in comparison to *T. rubrum*, with significantly decrease ($p < 0.01$) in the radial diameter. *T. schoienlinii* and *M. canison* were the most resistant dermatophyte to L- Glutamic acid. *T. verrcosum* was the most resistant to DL-Tryptophan while other dermatophytes suppressed at 1.0 mg/ml concentration. At cutoff concentration of 0.75 mg/ml, the sensitivity to DL-Tryptophan decreased respectively in *E. floccosum*, *T. schoienlinii* and *M. canison*,

and also equally in *T. rubrum* and *M. gypseum* by significant ($p < 0.01$) increase in the radial diameter. Except for *T. schoienlinii* and *M. canison* that were suppressed at 1.0 mg/ml level of L-Tyrosine, other four dermatophytes were resistant to this amino acid. The association of dermatophytes' sensitivity to other amino acids are shown in table 2.

Table 2 shows amino acids with low antidermatophytic property, and also the comparison results of dermatophytes' colony diameters at two amino acid concentrations of 0.1 mg/ml and 1.0 mg/ml. Weak inhibitory effects of amino acids occurred with L- Serine, L- Alanine, L- Lucine, L- Asparagine, L- Glutamine, L- Methionine, L- Arginine, L- Cysteine monochloride, L- Histidine monochloride, L-Glycine and L-Isoleucine. At two concentrations, most of amino acids did not show any significant difference ($p > 0.01$) in affecting some dermatophytes' colony diameters. However, others including L-Arginine, L-Lysine monohydrochloride, L-Methionine, L-Histidine hydrochloride, though not completely, had significant inhibitory effect ($p < 0.01$) on the growth of most dermatophytes shown in percent (% difference in growth at two amino acid concentrations). Table 3 compares the optimum concentrations of the effective amino acids for the growth of dermatophytes and the resultant colony diameters.

Table 2. Amino acids with less antidermatophytic property

Amino acids	Dermatophytes					
	<i>T. verrcosum</i>	<i>T. schoenleinii</i>	<i>T. rubrum</i>	<i>M. canison</i>	<i>M. gypseum.</i>	<i>E. floccosum</i>
L-Arginine	NE	40%↓	55%↓	15%↓	50%↓	58%↓
L-Histidine hydrochloride	NE	NE	NE	21%↓	10%↓	20%↓
L-Methionine	23%	31%↓	42%↓	20%↓	NE	NE
L-Glycine	NE	NE	22%	NE	NE	NE
L-Asparagine monohydrochloride	NE	NE	NE	NE	15%	NE
L-Phenylalanine	NE	NE	NE	43%	NE	NE
L-Proline	NE	NE	NE	NE	NE	NE
L-Hydroxyproline	NE	NE	NE	45%↓	12%↓	NE
L-Histidine	NE	NE	NE	NE	10%↓	18%↓
L-Threonine	NE		NE	NE	NE	NE
L-Lysine monohydrochloride	20%↓	28%↓	56%↓	NE	31%↓	46%↓
L-Leucine	NE	NE	NE	NE	NE	NE
L-Isoleucine	NE	NE	NE	20%	NE	NE
L-Alanine	NE	NE	NE	NE	NE	NE
L-Valine	NE	NE	NE	NE	NE	14%↓
L-Serine	22%↓	NE	NE	NE	NE	41%↓
L-Glutamine	NE	NE	NE	NE	28%↓	NE

%↓= percentage of dermatophytes growth inhibition by comparison of the mean colonies' diameter at amino acid concentrations of 1.0 mg/ml and (0.1 mg/ml) (p<0.01)
NE= no effective (p>0.01)

Table 3. Comparison of the optimum concentrations of the effective amino acids for the growth of the dermatophytes

Amino acid	Dermatophytes					
	<i>T. verrcosum</i>	<i>T. schoenleinii</i>	<i>T. rubrum</i>	<i>M. canison</i>	<i>M. gypseum.</i>	<i>E. floccosum</i>
	(Colony diameter) mm					
L-Cystein hydrochloride	0.1 (43.0±1)*	0.1 (24.7±0.6)	0.1 (43.3±1.5)	0.1 (48.3±1.2)	0.0 (52.3±1.5)	0.0 (16.7±0.6)
L-Cysteine	0.1 (21.3±0.6)	0.1 (26.7±0.6)	0.1 (45.0±1.7)	0.0 (47.0±1.7)	0.1 (52.0±3.1)	0.1 (18.00±1)
L-Aspartic acid	0.25 (18.6±2)	0.1 (24.3±0.6)	0.1 (42.3±2.1)	0.0 (47.0±1.7)	0.25 (55.0±4.4)	0.0 (16.7±0.6)
L- Glutamic acid	0.25 (18.0±1)	0.1 (22.3±1.2)	0.1 (43.0±1.0)	0.0 (47.0±1.7)	0.1 (52.3±2.1)	0.0 (16.7 ±0.6)
DL-Tryptophan	0.1 (17.00±3)	0.0 (22.3±1.2)	0.25 (48.0±2.7)	0.1 (49.3±2.5)	0.0 (52.3±1.5)	0.1 (16.8±0.6)
L-Tyrosine	0.1 (17. 7±1.2)	0.1 (23.7±0.6)	0.1 (43.0±1.7)	0.1 (48.0±1.7)	0.1 (59.0±1.5)	0.1 (17.0±0.6)
L-Arginine	0.0 (32.3±3)	0.1 (23.0±1.0)	0.0 (39.7±4.9)	0.1 (52.0±3.0)	0.0 (52.3±1.5)	0.0 (16.7±0.6)
L-Methionine	0.1 (36.7±3.7)	0.1 (23.7±1.2)	0.0 (39.7±4.9)	0.0 (47.0±1.7)	0.0 (52.3±1.5)	0.0 (16.7±0.6)
L-Lysine monohydrochloride	0.1 (35.0±2)	0.1 (26.0±1.0)	0.0 (39.7±4.9)	0.1 (51.0±2.0)	0.0 (52.3±1.5)	0.0 (16.7±0.6)

*The mean colony diameter±SD

Discussion

Due to the relatively high prevalence of dermatophytosis in Iran and occurrence of the side effects of antifungal compounds it seems important to find effective agents with minimal side effects and also to investigate different conditions for limiting the growth of dermatophytes. Therefore, in the present study antidermatophytic effects of 23 amino acids was investigated and compared among six Iranian dermatophytes. Six amino acids including L-Cysteine hydrochloride, L-Cysteine, L-Aspartic acid, L-Glutamic acid, L-Tyrosine and DL-Tryptophan indicated the most effective inhibitory property against six dermatophytes. Among these amino acids, L-Cysteine hydrochloride, L-Aspartic acid and L-Glutamic acid had acidic agents, L-Cysteine hydrochloride and L-Cysteine contained sulfur in their structure, and two amino acids including L-Tyrosine and LD-Tryptophan contained aromatic group. Therefore it is anticipated that sulfur, aromatic and acidic groups are responsible for antifungal property. L-Cysteine hydrochloride has both sulfur structure and acidic agent and its antifungal property is the most effective among all amino acids. L-Histidine and L-Histidine chloride indicated the same effects against all dermatophytes. Hence, chloride group may fail to have a remarkable role in antifungal property. Third sulfur containing amino acid (L-Methionine) showed slight antifungal activity. Kunert et al. used 38 chromomeric substances in their study, to investigate the specificity of the photolytic

enzymes of seven species of dermatophytes and three related keratinolytic soil fungi [21]. The best found substrates for photolytic enzymes were L-Phenylalanine, L-Leucine, L-Alanine, L-Methionine and L-Arginine (i.e. amino acids with hydrophobic or basic side chains) in the P1 position. Therefore the antifungal property of L-Methionine could be attributed to its sulfur group. Of the amino acids in the P2 position, L-Proline was the most effective in accelerating the hydrolysis of the respective substrate [21]. In India one study on *M. gypseum* and *T. mentagrophytes* showed that cysteine hydrochloride and L-Aspartic acid have inhibitory effects on both dermatophytes. The minimal inhibitory concentration of cysteine hydrochloride was 0.5 gr/dl for *M. gypseum* and 0.4 gr/dl for *T. mentagrophytes*. L-Aspartic acid with concentration of 1 gr/dl also decreased the growth of *M. gypsum* to 100% and the growth of *T. mentagrophytes* 48% [22]. In a study by Carrillo and colleagues, all the 24 under-study dermatophyton fungi grew poorly in the presence of cysteine. None of the strains, except *T. mentagrophytes* var. *quinckeanum*, grew in the presence of 0.04 M L-Cysteine. The strains, grown on a medium containing cysteine, showed morphological changes. The surface of the colonies lost its velvety appearance and became awnless or waxy [23, 24]. Pandey et al. reported that L-Cysteine hydrochloride and L-Aspartic acid show antifungal property toward *M. gypseum* and *T. mentagrophytes* but other effective

amino acids such as Glutamic acid, LD-Arginine and L-Tyrosine do not display such property. Cystatins are also able to inhibit *in vitro* fungal growth [22]. However, the *in vivo* antifungal effect of these inhibitors has not been previously tested. It is advised that such amino acids in the sweat of resistant and sensitive patients to dermatophytes be assayed and compared in order to determine their *in vivo* effects in pathogenesis of dermatophytes. On the other hand, Hashimoto et al. isolated gallerimycin, a cysteine-rich antifungal peptide from *Samia cynthia ricini* and proved its immunization effect on the worm. Cysteine exhibited a double effect against dermatophytes *in vivo*, one by direct antifungal effect and the other by immunizing the host [25]. Antifungal property of some yeasts such as *Pichia pastoris* and plants such as *Calotropis procera*, *Plumeria rubra*, *Carica candamarcensis* and *Euphorbia tirucalli* are attributed to cysteine [26]. LD-Arginine, an indolic amino acid, has an antifungal property against all studied Iranian dermatophytes except *T. verrucosum* and this is a unique property of this substance. It may be due to the existence of an enzyme such as argininease. Consonni et al. showed that LD-Arginine metabolites are required for antifungal defense in *Arabidopsis mlo2* mutant [27]. Chen et al. suggested that bacterial persistence could be controlled by Trp/Arg containing antimicrobial peptides [28]. Moreover, two amino acids containing acid groups, L- Glutamic acid and aspartic acid, have also antifungal property against all studied dermatophytes and this may be related to their acid group. In a study on

antifungal property of *Scutellaria baicalensis*, Sheng et al. found that 14.62% structure of this plant consists of 18 amino acids, and among the amino acids, L- Aspartic acid, L- Glutamic acid and L- Leucine are significantly high in the leaves [29]. Another study indicated the role of aspartic protease inhibitors as potential anti-candida albicans drugs [30]. Bakhshi et al. analyzed the levels of different amino acids in stratum corneum and plasma of the patients with dermatophytosis and compared them with normal subjects. They reported that increase in Glutamates- Asparagine- Histidine- Glutamine- Arginine- Citrulline - Threonine- Methionine - Leucine - Ornithine levels in patients with dermatophytosis may make them susceptible to dermatophytes growth. However, the *in vivo* fungal effects of these amino acids have not been individually tested [16]. More *in vivo* studies are needed to investigate the effects of different levels of amino acids, individually and together, on dermatophyte growth and degradation of human skin.

Conclusions

The amino acids cysteine hydrochloride, L- Cysteine, L- Aspartic acid, L- Glutamic acid, LD-Arginine and L- Tyrosine have antifungal property against the studied dermatophytes. By using the properties of these effective amino acids, antifungal drugs may be synthesized at lower cost and less side effects against different dermatophytes.

Conflict of Interest

The authors declare that they have no competing of interests.

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