Antioxidative enzymes and increased oxidative stress in depressive women

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Abstract

Objectives: To investigate the activities of the main antioxidative enzymes and oxidative stress in women with depressive disorder (DD).

Methods: In 35 drug-naive women with DD and 35 age matched healthy women enzymes superoxide dismutase (CuZnSOD), catalase (CAT), glutathione peroxidase (GPX1), glutathione reductase (GR) and paraoxonase (PON1), concentrations of conjugated dienes (CD), reduced glutathione (GSH) and anthropometric and clinical data were investigated.

Results: Women with DD were found to have decreased activities of GPX1 (p<0.05), decreased concentrations of GSH (p<0.05), and increased activities of GR (p<0.05), CuZnSOD (p<0.001), and concentrations of CD (p<0.05). Activity of GPX1 was positively correlated with concentration of GSH (p<0.05). Concentrations of CD were positively correlated with TG (p<0.01).

Conclusion: Our set of depressive women was characterized by changes indicating an increased oxidative stress, as well as by certain features of metabolic syndrome.

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Keywords: Depressive disorder; Oxidative stress; Antioxidative enzymes; Conjugated dienes

Introduction

Depressive disorder (DD) belongs to diseases, incidence of which is now increasing all around the world. In the USA, it was established, that about 16% of the population fall ill with major depressive disorder during the lifetime [1]. In Finland, 5% prevalence of the depression was described [2]. In 2006, 168 new cases of affective disorders per 100,000 inhabitants were noticed in the Czech Republic, the incidence being 2 times higher in women than in men [3]. The dysfunction of serotonergic, noradrenergic and dopaminergic neurotransmission [4,5], abnormal regulation in the hypothalamic–pituitary–adrenal axis (HPA) [6], disturbance of cellular plasticity including reduced neurogenesis [7], or chronic inflammation, connected with higher oxidative stress [8] could play a role in the pathogenesis of DD.

Large consumption of oxygen (up to 20% of the total requirement of organism), high amount of polyunsaturated fatty acids, which are prone to oxidation, high amount of iron and low activities of antioxidant enzymes contribute to higher sensitivity of brain to oxidative stress [9]. Oxidative stress is defined as the imbalance between production of reactive oxygen and nitrogen species (RONS) and their insufficient decomposition by the antioxidative system [10]. This defence system involves enzymatic antioxidants — superoxide dismutase (EC 1.15.1.1.; SOD), glutathione peroxidase (EC 1.11.1.9; GPX), glutathione reductase (EC 1.6.4.2; GR), catalase (EC 1.11.1.6; CAT) and paraoxonase (EC 3.1.8.1; PON) as well as non-enzymatic antioxidants — reduced glutathione (GSH), provitamin A, vitamin C and E, coenzyme Q10, carotenoids and trace elements like copper, zinc or selenium. Increased production of RONS has been observed in patients with neurodegenerative and psychiatric diseases such as Alzheimer’s and Parkinson’s.
disease or schizophrenia [11–13]. Neurodegenerative changes, which are augmented by inflammation and oxidative stress, play an important role also in the pathogenesis of the DD [14,15]. The raised level of oxidative stress is supposed to be one of the factors, standing behind higher incidence of type 2 diabetes mellitus (DM2) and cardiovascular diseases (CVD), which were observed in patients with depression [16,17]. However, only few studies have studied an oxidative stress in DD and the results have been inconsistent. The aim of this study was to determine the activities of main antioxidative enzymes, concentrations of reduced glutathione and conjugated dienes (CD) as marker of lipoperoxidation, and their relations to anthropometric and selected metabolic parameters in women with DD in comparison with healthy controls.

Methods

Subjects

Thirty five women with DD, recruited from the consecutive outpatients of the Psychiatric Department of 1st Faculty of Medicine of Charles University in Prague from May 2006 to May 2008, and 35 age-matched healthy controls were included in the study. Depressive disorder was diagnosed according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, DSM-IV (American Psychiatric Association, 1994) [18]. All patients were evaluated using Hamilton Depression Rating Scale (HAM-D).

Exclusion criteria of the study were: history of cardiovascular and cerebrovascular disease, DM, hepatic and/or renal diseases, hypothyroidism, malignancies, macroalbuminuria (proteinuria higher than 300 mg/day), excessive alcohol consumption (>30 g/day), treatment with hypolipidemic medications, supplementation by vitamins, polyunsaturated fatty acids and/or antioxidants. Patients have completed the 7 days dietary questionnaire. Food intake was processed by the software NutriMaster. We have evaluated intake of total energy, protein, fat, carbohydrate, vitamins and minerals. The study protocol was approved by the Joint Ethical Committee of the General Teaching Hospital and the 1st Faculty of Medicine of Charles University in Prague. Written informed consent was obtained from all participants.

Blood samples

Blood samples were obtained after overnight fasting. Activities of CAT, GR, GPX1 and CuZnSOD were measured in haemolysed erythrocytes. The blood samples were collected into the tubes with K2 EDTA, erythrocytes were washed three times with a NaCl isotonic solution (9 g/L). Serum was used for the determination of all other parameters. The samples were stored at −80 °C until assay. The haemotological parameters were measured by routine laboratory techniques using an autoanalyzer (Coulter LH750 — haematological analyzer, Beckman Coulter).

Measurement of enzyme activities

Glutathione peroxidase 1

The activity was measured by the modified method of Paglia and Valentine using tert-butyl hydroperoxide as a substrate [19]. Briefly, 580 μL of 172.4 mM tris–HCl buffer containing 0.86 mM EDTA, pH=8.0; 100 μL of 20 mM GSH, 100 μL of 10 U/mL GR, 100 μL of 2 mM NADPH and 100 μL of diluted sample were pipetted into the cuvettes. The reaction was started after 10 min of incubation at 37 °C by the addition of 20 μL of 9.99 mM tert-butyl hydroperoxide. The rate of NADPH degradation was monitored spectrophotometrically at 340 nm. Blank was run for each sample. Activity of GPX1 was calculated using the molar extinction coefficient of NADPH 6220 M−1 cm−1 and expressed as U/g haemoglobin. One unit of GPX1 (U) is defined as 1 μmol of NADPH oxidized to NADP per minute.

Glutathione reductase

The activity was measured according to the method of Goldberg et al. [20]. Briefly, 700 μL of 0.127 M potassium phosphate buffer containing 0.633 mM Na2EDTA·2H2O, pH=7.2 was added to cuvettes followed by 100 μL of 22 mM oxidized glutathione (GSSG) and 100 μL of diluted sample. The reaction was started after 10 min of incubation at 37 °C by addition of 100 μL of 1.7 mM NADPH. The rate of NADPH degradation was monitored spectrophotometrically at 340 nm. Blank was run for each sample. Activity of GR was calculated using the molar extinction coefficient of NADPH 6220 M−1 cm−1 and expressed as U/g haemoglobin. One unit of GR (U) is defined as the amount of enzyme catalyzing the reduction of 1 μmol of GSSG per minute.

Catalase

The activity was determined by the modified method of Aebi [21]. The reaction mixture in cuvettes contained 876 μL of 50 mM potassium phosphate buffer, pH=7.2 and 25 μL of diluted sample. The reaction was started after 10 min of incubation at 30 °C by addition of 99 μL of 10 mM H2O2. The rate of H2O2 degradation was monitored spectrophotometrically at 240 nm. Blank was run for each sample. Catalase activity was calculated using the molar extinction coefficient of H2O2 43.6 M−1 cm−1 and expressed as kU/g haemoglobin. One unit of CAT (U) is defined as 1 μmol of H2O2 decomposition per minute.

CuZn-Superoxide dismutase

The activity was determined according to the modified method of Štipek et al. [22]. The reaction mixture in cuvettes contained 700 μL of 50 mM potassium phosphate buffer, pH=7.2; 50 μL of xanthine oxidase; 100 μL of NBT and 50 μL of diluted sample. The reaction was started after 10 min of incubation at 25 °C by addition of 100 μL of 1 mM xanthine. The rate of NBT-formazan generation was monitored spectrophotometrically at 540 nm. Blank was run for each sample. Superoxide dismutase activity was calculated by means of calibration curve and expressed as U/g haemoglobin. One unit
of SOD (U) is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. Superoxide dismutase standard (Cat. No. S9636-1KU) was purchased from Sigma Aldrich (St. Louis, MO USA).

**Paraoxonase 1**

The arylesterase activity of PON1 was measured according to the method of Eckerson et al. using phenylacetate as a substrate [23]. Briefly, 900 μL of 20 mM Tris–HCl buffer containing 1 mM CaCl₂, pH=8.0 was added to cuvettes followed by 50 μL of diluted serum sample. The reaction was started by addition of 50μL of 100 mM phenylacetate. The rate of phenol generation was monitored spectrophotometrically at 270 nm. Blank was run for each sample. Arylesterase activity of PON1 was exhibited 50% dismutation of the superoxide radical. Superoxide dismutase standard (Cat. No. S9636-1KU) was purchased from Sigma Aldrich (St. Louis, MO USA).

**Measurement of concentration of reduced glutathione**

Reduced glutathione was measured by the modified spectrophotometric method according to Griffith [24]. Suspension of washed erythrocytes (500 μL) was mixed with 100 μL of diluted acetic acid in water (6%, v/v), haemolysate was vortexed and 400 μL of S-sulphosalicylic acid 10% (w/v) was immediately added. After centrifugation at 10 000 g for 2 min, supernatant solution was collected for analysis. This method is based on the determination of relatively stable product of reduction of 5.5’-dithiobis-2-nitrobenzoic acid (DTNB) reduction by sulfhydryl compounds to yellow product. Briefly, 50 μL of 0.125 M potassium phosphate buffer containing 1 mM CaCl₂, pH=8.0 was added to micro-cuvettes followed by 37.5 μL of the sample and 12.5 μL of 6 mM DTNB. The absorbance of the yellow product (reduced chromogen) was measured at 412 nm. Concentration was calculated by means of calibration curve and was expressed as μg/g haemoglobin.

**Measurement of concentration of conjugated dienes**

Serum low density lipoproteins were isolated by precipitation method of Ahotupa et al. [25]. Concentrations of CD in precipitated LDL were measured by the modified method of Wieland et al. [26]. Serum samples were stabilized with EDTA (10:1 v/v) and analyzed within 2 weeks. The precipitation buffer consisted of 0.064 M trisodium citrate adjusted to pH 5.05 with 5 M HCl, and contained 50,000 IU/L heparin. Sample (110 μL) of serum with EDTA (10:1 v/v) was added to 1 mL of the heparin-citrate buffer. After mixing, the suspension was incubated for 10 min at room temperature. The precipitated lipoproteins were then separated by centrifugation at 2800 rpm for 10 min. Supernatant was removed and the pellet was resuspended in 100 μL of NaCl isotonic solution (9 g/L); this process, individual for each sample, did not exceed 3 s to prevent LDL oxidation. Lipids were extracted by chloroform–methanol (2:1), the mixture was incubated for 10 min with intermittent mixing, 250 μL redistilled water was used for phase separation. The mixture was centrifuged at 3000 rpm for 5 min. The 800 μL of lower layer (infranatant) was dried under nitrogen, redissolved in 300 μL of cyclohexane, and analyzed spectrophotometrically at 234 nm. The concentration of CD was calculated using the molar extinction coefficient 2.95 × 10⁴ M⁻¹ cm⁻¹ and expressed as mmol/L serum.

**Statistical analysis**

All data were expressed as median (25th–75th percentiles). Normality of distribution of data was tested with Shapiro–Wilks W test. Differences between compared groups were tested with one-way ANOVA. Mann–Whitney U test was used for non-parametric comparison of groups. The Spearman correlation coefficients were used for correlation analysis. All statistical analyses were performed using version 8.0 of Statsoft software Statistica (2007, CZ).

**Results**

The basic characteristics and essential biochemical parameters observed in the studied groups are shown in Table 1, parameters of oxidative stress are presented in Table 2. Patients with DD had significantly raised values of waist circumference, TG, glucose and index of insulin resistance (HOMA-IR) in comparison with control group. The mean systolic and diastolic blood pressure (SBP and DBP) did not differ significantly. There were also no significant differences in concentrations of HDL-C, LDL-C, CRP, apo A-I and apo B, as well as those of calcium, zinc and copper. We have found no statistical

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Subject characteristics.</th>
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<tbody>
<tr>
<td></td>
<td>Depression</td>
</tr>
<tr>
<td>N (female)</td>
<td>35</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64.5 (50.0–75.1)</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>26.1 (24.1–29.4)</td>
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<tr>
<td>Waist (cm)</td>
<td>87.0 (77.0–96.0)</td>
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<tr>
<td>Systolic BP (mm Hg)</td>
<td>120.0 (120.0–135.0)</td>
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<tr>
<td>Diastolic BP (mm Hg)</td>
<td>80.0 (70.0–80.0)</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>5.42 (4.55–6.57)</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.32 (0.95–1.8)</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.42 (1.24–1.71)</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>3.14 (2.54–4.05)</td>
</tr>
<tr>
<td>Apo A-I (g/L)</td>
<td>1.41 (1.26–1.56)</td>
</tr>
<tr>
<td>Apo B (g/L)</td>
<td>1.02 (0.86–1.34)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.0 (4.6–5.9)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.22 (1.97–2.67)</td>
</tr>
<tr>
<td>CRP (mmol/L)</td>
<td>3.3 (2.0–7.9)</td>
</tr>
<tr>
<td>Ca (mmol/L)</td>
<td>2.35 (2.29–2.47)</td>
</tr>
<tr>
<td>Cu (mmol/L)</td>
<td>21.3 (17.8–23.5)</td>
</tr>
<tr>
<td>Zn (mmol/L)</td>
<td>15.2 (13.8–16.7)</td>
</tr>
<tr>
<td>Cu/Zn</td>
<td>1.33 (1.15–1.64)</td>
</tr>
</tbody>
</table>

Abbreviations used: BMI: body mass index, BP: blood pressure, TC: total cholesterol, TG: triglycerides, HDL-C: high density lipoprotein, LDL-C: low density lipoprotein, CRP: C-reactive protein; Data were expressed as median (25th–75th percentiles). Statistical analysis: * p<0.05; ** p<0.01.
significant differences in nutritional habits between women with DD and control group (data not shown).

Erythrocyte activities of GR and CuZnSOD and concentrations of CD in precipitated LDL were increased in depressive women; however, activities of GPX1 were decreased. Reduced glutathione was significantly lower in depressive women than in the control group. Activities of CAT and PON1 were not altered in patients with DD.

In women with DD, activities of PON1 were positively correlated with concentrations of HDL-C \((r=0.457, p<0.01)\), apo A-I \((r=0.379, p<0.05)\) and calcium \((r=0.371, p<0.05)\), but in control group we have found only positive correlation with apoA-I \((r=0.492; p<0.05)\). Furthermore, activities of CuZnSOD were positively correlated with concentrations of zinc in DD (Fig. 1) and also in control group \((r=0.393, p<0.05; r=0.477, p<0.05, \text{ respectively})\). There was no significant correlation of CuZnSOD with copper in both groups.

Activities of GPX1 were positively correlated with concentrations of GSH \((r=0.284, p<0.05)\) in DD, but not in control group. There were no correlations observed between activities of individual antioxidant enzymes.

Concentrations of serum TG were positively correlated with concentrations of CD in precipitated LDL in the DD group (Fig. 2) and in the control one \((r=0.480, p<0.01; r=0.391; p<0.05, \text{ respectively})\). We did not find any correlation between HAM-D score and any of observed parameters.

**Discussion**

The most important findings of this study were significantly increased concentrations of CD in precipitated LDL, indicating increased lipid peroxidation, accompanied by the decrease in activity of GPX1 and increase in activities of both CuZnSOD and GR in women with DD. The presence of IR and certain features of metabolic syndrome (MetS) in our set of women with DD were further important findings.

Oxidative stress was accepted to participate in the pathophysiology of neurodegenerative conditions such as Alzheimer’s disease [27,28], HIV-associated dementia [29], Parkinson’s disease [30]. Neurodegenerative changes of brain have been demonstrated in patients with DD, in which also markers of oxidative stress were previously described, such as altered activities of antioxidative enzymes and increased lipid peroxidation products [31–34].

Glutathione peroxidase is ubiquitous enzyme responsible for the degradation of lipid hydroperoxides and of \(\text{H}_2\text{O}_2\) to hydroxyderivates and water. Decreased activities of GPX1 in erythrocytes were found in our depressive patients, similarly as in the study of Ozcan et al. [35], who described lower activities of GPX1 in patients with affective disorders in comparison with healthy controls. However, Bilici et al. [33] found increased

### Table 2

<table>
<thead>
<tr>
<th>Parameters of oxidative stress.</th>
<th>Depression</th>
<th>Controls</th>
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<tbody>
<tr>
<td>GPX1 (U/g Hb)</td>
<td>53.7 (42.7–65.7)²</td>
<td>64.0 (52.9–70.7)</td>
</tr>
<tr>
<td>GR (U/g Hb)</td>
<td>7.95 (6.84–8.62)²</td>
<td>7.00 (6.19–8.30)</td>
</tr>
<tr>
<td>GSH (µg/g Hb)</td>
<td>568.75 (387.93–3484.01)²</td>
<td>2374.93 (515.16–5668.35)</td>
</tr>
<tr>
<td>CuZnSOD (U/g Hb)</td>
<td>2356.2 (2080.75–2586.5)+++</td>
<td>1930.5 (1309.2–2249.7)</td>
</tr>
<tr>
<td>CAT (kU/g Hb)</td>
<td>174.0 (155.2–217.9)</td>
<td>189.0 (166.6–215.4)</td>
</tr>
<tr>
<td>PON1 (kU/L)</td>
<td>161.3 (140.8–196.2)</td>
<td>175.9 (146.2–207.3)</td>
</tr>
<tr>
<td>CD (mmol/L)</td>
<td>55.7 (47.7–80.8)²</td>
<td>53.3 (43.8–62.1)</td>
</tr>
</tbody>
</table>

Abbreviations used: GPX1: glutathione peroxidase1, GR: glutathione reductase, GSH: reduced glutathione, CuZnSOD: CuZn-superoxide dismutase, CAT: catalase, PON1: paraoxonase1, CD: conjugated dienes, Hb: haemoglobin; Data were expressed as median (25th–75th percentiles). Statistical analysis: \(^* p<0.05; ^{++} p<0.01; ^{+++} p<0.001\).
activities of GPX1 in erythrocytes of patients with major depression, whereas Andrezza et al. [36] did not find any significant changes in patients with bipolar disorder. Activity of GPX1 could be decreased due to lower concentration of its substrate — GSH that we have found in women with DD. Reduced glutathione is one of the most important intracellular antioxidants in the cell and is enzymatically oxidized to GSSG in a number of biochemical pathways. In the present study we have observed significantly decreased concentrations of GSH in depressive women compared to control. To our knowledge, there has been no clinical study regarding data on GSH concentrations in patients with depressive disorders. The observed decrease of GSH were also described in patients with autism [37,38], schizophrenia [39] and Down syndrome [40] have reduced levels of total GSH. Reduced glutathione reacts also nonenzymatically with RONS leading to the glutathiol radical that reacts with further GSH to GSSG radical anion formation. Oxidized glutathione radical anion is involved in the conversion of oxygen to superoxide. The conversion of GSSG back to GSH is catalyzed by GR. In our study, we have found increased activities of GR in erythrocytes. Bilici et al. [33] described raised activities of GR in plasma, but no significant differences in erythrocytes in patients with major depression.

Studies have described a variety of intracellular sources of superoxide that include nitric oxide synthase, xanthine oxidase, cyclooxygenase and NADPH oxidase [41–44]. The most important source of superoxide in vascular cells is NADPH oxidase [45]. Decomposition of superoxide into H2O2 is catalyzed by SOD. We have found increased CuZnSOD activities in erythrocytes of depressive patients compared with healthy persons, similarly to Sarandol et al. [32]. They suggested that CuZnSOD activity is increased in response to increased ROS production. Bilici et al. [33] have also observed increased CuZnSOD activity in erythrocytes of depressive patients. Inconsistent results were published for serum CuZnSOD activities. Herken et al. [34] have found decreased, whereas Khanzode et al. [46] elevated CuZnSOD activities in patients with major depression. We have found positive correlation between CuZnSOD activity and concentration of zinc, which is responsible for the stability of CuZnSOD structure as its cofactor [47].

Activities of CAT in erythrocytes were not altered in our set of women with DD, in accordance with Bilici et al. [33]. However, Szuster-Ciesielska et al. [48] found raised activities of CAT in serum of patients with major depression and Ozcan et al. [35] described decreased CAT activities in erythrocytes of patients with affective disorders. Induction of CAT or SOD does not necessarily lead to the induction of the other one [49]. The increased activity of SOD leads to increased amounts of hydrogen peroxide that is then degraded by GPX in its low concentrations and by CAT in its high concentrations [50]. It could be supposed that the concentration of hydrogen peroxide wasn’t enough high to increase activity of CAT, and that the task of H2O2 degradation remains on GPX. But GPX activity is dependent on GSH, as its substrate. This antioxidant is rapidly consumed in oxidative stress. It is problematic whether GPX could function appropriately in low GSH concentrations.

The activities of PON1 were not altered in women with DD, as well as levels of apo A-I, HDL-C and calcium. Apolipoprotein A-I plays a key role for PON1 because of the connection of PON1 to HDL is through apo A-I. We have found positive correlation between PON1 activity and both apo A-I and HDL- C concentrations. Paraoxonase is calcium dependent enzyme; calcium is located in the active site of enzyme. It is consistent with our finding of a positive correlation between the PON1 activity and calcium concentrations in patients with DD.

Increased concentrations of CD in LDL indicate an elevation of minimally modified (oxidized) LDL in vivo. Raised concentrations of CD in LDL were found in insulin-resistant states such as MetS and DM2 [51–53], however, different results were published by Gavella et al. [54].

Observed hypertriglyceridemia (HTG) and higher glycemia, the accumulation of visceral fat and IR could play a role in changes of oxidant/antioxidant balance in our set of depressive women. In nondiabetic human subjects, both BMI and waist circumference were closely correlated with the markers of systemic oxidative stress (plasma TBARS, urinary 8-epi-PGF2α) [55]. Hypertriglyceridemia was associated with an increased oxidative stress in experimental rats [56] and also in humans [57]. Inconsistent results were obtained with regard to the activities of antioxidant enzymes in insulin-resistant states. In one study, increased activity of CAT, decreased of GPX and non-changed of SOD was found in type 2 diabetic patients [58] while in another study [59] the activities of GPX, SOD and CAT in red blood cells were significantly decreased in diabetic subjects when compared with healthy controls. Some authors suggest decreased GPX1 activity as cardiovascular risk factor that was in the prospective study associated with increased extent of atherosclerotic lesions [60].

In summary, we have found significant increase in CuZnSOD and GR activity and simultaneous decrease of GPX1 activity as well as elevated concentrations of CD in precipitated LDL, which positively correlated with TG in our set of depressive women. These findings are in accordance with hypothesis that oxidative stress may play an important role in the pathogenesis of depression. Metabolic changes and markers of IR in women with DD suggest the relationships between MetS and DD. Increased oxidative stress could be a possible connection between depression, IR and increased incidence of both DM2 and CVD.

Acknowledgments

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