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Absorption of Aminoethyl Cysteine Ketimine Decarboxylated Dimer in Mice: Effect on Plasma Antioxidant Potential

Alessandro Piazzon,[†] Alberto Macone,[‡] Rosa Marina Matarese,[‡] Alberto Finamore,[†] and Mirella Nardini*[,†]

[†]National Institute for Food and Nutrition Research, Via Ardeatina 546, 00178, Rome, Italy

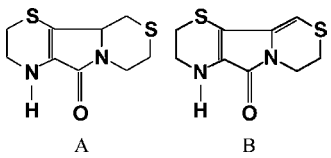
[‡]Department of Biochemical Sciences "A. Rossi Fanelli", University of Rome, "La Sapienza", 00185, Rome, Italy

ABSTRACT: Aminoethylcysteine ketimine decarboxylated dimer (AECK-DD) is a natural compound with antioxidant properties of a new family of sulfur-containing amino acids. It has been detected in human urine and plasma, in mammalian cerebellum, and in dietary vegetables. In this study, we first demonstrate the absorption of AECK-DD in mice from AECK-DD-supplemented diet, using both liquid chromatography with electrochemical detection and gas chromatography coupled with mass spectrometry. AECK-DD circulates in the plasma of supplemented mice at a micromolar concentration and is incorporated in liver tissue. The absorption of AECK-DD is dose dependent. The dehydrogenation product of AECK-DD was also identified in plasma and liver of mice fed the AECK-DD-supplemented diet. A significant increase in plasma antioxidant potential was measured in mice fed AECK-DD-supplemented diet with respect to mice fed the control diet. These results demonstrate for the first time the absorption of AECK-DD from diet and the physiological relevance of this compound through its antioxidant action in vivo.

KEYWORDS: *aminoethylcysteine ketimine decarboxylated dimer, antioxidant, plasma, liver, mouse*

INTRODUCTION

In the past decade, sulfur-containing compounds, such as sulforaphane and ajoene, have received renewed attention due to their antioxidant properties and biological functions.[1−6] Organosulfur compounds have been shown to exhert various biological effects such as antioxidant effects, anti-inflammatory properties, inhibition of platelet aggregation, anticarcinogenic activity, and reduction of cholesterol levels and blood pressure.[1−6] Recently, a new family of sulfur-containing compounds has been described.[7,8] Among these, aminoethylcysteine ketimine decarboxylated dimer (AECK-DD) (Figure 1) is a natural sulfur-containing tricyclic compound,



A B

Figure 1. Chemical structures of AECK-DD (A) and its dehydrogenated form (B).

detected in human plasma at a micromolar concentration,[9] in human urine,[10] in mammalian cerebellum,[11] and in dietary vegetables.[12] Recently, it was also identified in the brain of cysteamine-treated rats.[13] AECK-DD also has been described to be associated with lipoproteins isolated from human plasma.[9] AECK-DD has a strong antioxidant activity.[8] It has been reported to scavenge reactive oxygen and nitrogen species (hydrogen peroxide, superoxide anion, hydroxyl radical, peroxynitrite, and its derivatives) in vitro, to inhibit copper-induced oxidation of human low-density lipoprotein,[14] and to protect human monocytic cells from oxidative stress induced by *tert*-butyl hydroperoxide.[15] Its antioxidant activity has been described to be comparable to that of α-tocopherol and more potent than ascorbic acid and glutathione.[15] Cysteic acid and taurine have been identified among the oxidation products of AECK-DD.[16−18] Recently, a dehydrogenation product arising from the oxidation of AECK-DD has been described.[19]

In spite of its widespread presence in biological samples, a biochemical route leading to the in vivo synthesis of this molecule has not been demonstrated so far. Recently, the presence of AECK-DD in some common edible vegetables present in the human diet has been reported,[12] suggesting a dietary supply of this compound.

This study was designed to examine whether exogeneous AECK-DD from the diet is absorbed in mice. Mice fed AECK-DD-supplemented diets (range, 0.05−0.2%) were analyzed for their plasma and liver AECK-DD contents at various interval times (up to 10 days), using both liquid chromatography with electrochemical detection and gas chromatography coupled with mass spectrometry (GC-MS). The effect of AECK-DD supplementation on plasma antioxidant potential was also studied, with respect to mice fed the control, unsupplemented diet.

MATERIALS AND METHODS

Chemicals and Reagents. AECK-DD was synthesized according to Fontana et al.[8] All chemicals and reagents were of analytical grade. Distilled water was purified using a Milli-Q water purification system (Millipore, Molsheim, France). Supelclean LC-SAX solid-phase

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extraction (SPE) cartridges (1 mL tubes) were from Supelco (Bellefonte, PA). Trolox, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and potassium peroxodisulfate were from Sigma-Aldrich (St. Louis, MO).

Diet and Animals. Forty Balb/c mice [initial weight, 23.3 ± 3.1 g; Charles River, Calco (LC), Italy] were individually housed in wire-bottom stainless-steel cages under controlled lighting. The animals were randomly divided into groups of five animals and fed for the specified experimental period a basal standard 4RF21 diet (Mucedola, Milano, Italy) supplemented with 0.05 or 0.2% (w/w) AECK-DD corresponding to 3.5 and 14.0 mg AECK-DD/day/mouse, respectively. Control groups were fed the basal diet without AECK-DD supplementation. The diets were prepared weekly and stored at 4 °C. Food and water were provided ad libitum. At the end of the experimental period, animals were anesthetized with pentobarbital injection (10 mg), blood was drawn via cardiac puncture, and the liver was excised and placed in cold phosphate-buffered saline (PBS). No significant differences in food intake, weight gain, final weight, and relative liver weight were detected among animals fed the different diets. Animal studies were performed under conditions approved by the National Health Ministry (Department of Food, Nutrition and Public Animal Health).

High-Performance Liquid Chromatography (HPLC) Instrumentation. The HPLC system consists of a Perkin-Elmer Series 4 liquid chromatograph (Perkin-Elmer, Norwalk, CT) with a gradient pump, column thermo-regulator, and autosampling injector (Gilson, Beltline, Middleton, WI) equipped with an electrochemical coulometric detector (ECD) (Coulochem II, ESA, Bedford, MA). Totalchrom chromatography workstation software was used for data processing. Operating conditions were as follows: column temperature, 30 °C; flow rate, 1 mL/min; injection volume, 100 μL; sensitivity range, 200 nA; applied potential, +600 mV; and filter, 2 s. Chromatographic separations were performed as already reported[20] on a Supelcosil LC-18 C_{18} column (5 μm particle size, 250 mm × 4.6 mm i.d.) including a guard column. Mobile phases A and B were employed. Solution A contained glacial acetic acid in water (1.25%); solution B was absolute methanol (MeOH). Isocratic elution was used with 70% A, 30% B at 1 mL/min flow (retention time, 31.0 min). A standard stock solution of AECK-DD (1 mg/mL in ethanol) was stored at −80 °C and used within 1 week. For the calibration curve, progressive dilutions in solution A of the stock solution were analyzed in the range 2.5−300 ng/mL. The detection limit for AECK-DD is 0.01 ng/mL. For quantitative determination, peak areas in the sample chromatograms were correlated with the concentrations according to the calibration curve. Prior to HPLC analysis, all samples were filtered using Millex-HV filters (Millipore, Bedford, MA) with 0.45 μm pore size.

GC-MS Analyses. GC-MS analyses were performed on a Hewlett-Packard 5970A MSD system (Hewlett-Packard, Palo Alto, CA). Chromatographic separations were carried out on a 30 m × 0.25 mm i.d. fused-silica capillary column coated with cross-linked 5% phenylmethyl siloxane, a film thickness of 0.25 μm, as the stationary phase (Hewlett-Packard); injection mode, splitless at a temperature of 260 °C. The column temperature program was as follows: 100 °C (1 min), then to 280 °C at a rate of 15 °C/min, and held for 8 min. The carrier gas was helium at a constant flow of 1 mL/min. The spectra were obtained in the electron impact mode at 70 eV ionization energy; ion source, 280 °C; and ion source vacuum, 10^{-5} Torr. MS analysis was performed using Agilent MSD productivity Chemstation that enables collection of both selected ion monitoring (SIM) data and scan data (mass range scan from m/z 50 to 500 at a rate of 0.42 scans s^{-1}) in a single run. For GC-SIM-MS analysis, three characteristic ions were selected for both AECK-DD (m/z 228, m/z 200, and m/z 154) and dehydro-AECK-DD (m/z 226, m/z 211, and m/z 180).

AECK-DD Measurements in Plasma, Liver Samples, and Diet by HPLC-ECD. Plasma was obtained from blood collected in ethylenediaminetetraacetic acid (EDTA) by 20 min of centrifugation at 1000*g* and 4 °C and was used immediately. Livers were homogenized in PBS at 10% (w/v), and homogenates were centrifuged 5 min at 15000*g*. Plasma and liver samples were treated for AECK-DD extraction essentially as previously described.[9] MeOH (1 mL) and chloroform (2 mL) were added to 0.1 mL of plasma or liver homogenate sample and 0.4 mL of twice-distilled water, saturated with NaCl. The resulting mixture was vortexed vigorously for 5 min and then centrifuged at 2000*g* for 5 min. The chloroform fraction was removed, and the sample was extracted with an additional 2 mL of chloroform, vortexed for 5 min, and centrifuged at 2000*g* for 5 min. The two organic layers were combined and evaporated and dried under nitrogen flow. The residue was dissolved with 0.5 mL of 2% MeOH in water, vortexed for 4 min, and passed through the LC-SAX tube (preconditioned with 1 mL of absolute MeOH and 2 mL of water). The tube was washed with 0.6 mL of water to complete elution. The eluate was filtered prior to HPLC-ECD analysis. The recovery of AECK-DD tested by the spiked method, by adding known amounts of standard AECK-DD to plasma or tissue homogenate samples before extraction, ranged from 98.7 to 110.5%.[9,20] For diet analysis, samples of 500 mg of pulverized basal diet suspended in 0.5 mL of deionized water saturated with NaCl were extracted and analyzed for their endogeneous AECK-DD content as above-reported.

GC-MS Analyses of Plasma, Liver, and Diet Samples. Plasma samples were treated for AECK-DD extraction essentially as previously described.[9] MeOH (1 mL) and chloroform (2 mL) were added in an extraction tube containing 0.1 mL of plasma sample and 0.4 mL of twice-distilled water saturated with NaCl. The resulting mixture was vortexed vigorously for 5 min and then centrifuged at 2000*g* for 5 min. The chloroform fraction was removed, and the sample was extracted with additional 2 mL of chloroform, vortexed for 5 min, and centrifuged at 2000*g* for 5 min. The two organic layers were combined and evaporated and dried under nitrogen flow. The residue was dissolved in 0.2 mL of dichloromethane and injected in the chromatograph without derivatization (injection volume, 1 μL). Liver samples (0.1 g) were homogenized in 0.5 mL of twice-distilled water and centrifuged at 2000*g* for 5 min. The supernatant was saturated with NaCl, added with MeOH (1 mL), and extracted twice with chloroform as described for plasma samples. The control diet was analyzed for AECK-DD content as described above. The diet (250 mg) was homogenized in 0.5 mL of twice-distilled water and centrifuged at 2000x g for 5 min. The supernatant was saturated with NaCl, added with MeOH (1 mL) and extracted twice with chloroform. The organic layers were dried under nitrogen flow and dissolved in 0.05 mL of dichloromethane, and aliquots were injected in the chromatograph without derivatization.

Antioxidant Activity Measurements by ABTS Assay. Standard AECK-DD was tested for its antioxidant activity with the ABTS method.[21] Briefly, a 1:1 (v/v) mixture of ABTS (7 mmol/L) and potassium persulfate (2.45 mmol/L) was left to stand for 16 h at room temperature (RT) in the dark to form radical cation $ABTS^{\bullet+}$. A working solution was diluted to absorbance values between 1.2 and 1.3 AU at 734 nm with twice-distilled water and equilibrated at 30 °C. Standard AECK-DD (0−25 nmol) was mixed with the working solution and diluted up to 1000 μL with twice-distilled water. The decrease of absorbance was measured at 734 nm, 30 °C, exactly 20 s after initial mixing and up to 6 min. Blanks were run substituting twice-distilled water to AECK-DD sample. Trolox (0−15 nmol) was used as a calibrating standard. The percentage inhibition of absorbance at 734 nm is calculated and plotted as a function of the concentration of the antioxidant tested (AECK-DD and trolox). The antioxidant activity of plasma samples was measured on 4 μL plasma aliquots as above-reported, and the percentage inhibition of absorbance at 734 nm was calculated. Again, blanks were run substituting twice-distilled water to plasma sample. All measurements were made in triplicate.

Statistical Analyses. Data presented are means ± standard errors. Statistical analyses for multiple comparisons were performed by one-factor analysis of variance (ANOVA; Fisher method). Student's t test was used for regression analyses. The probability of $p < 0.05$ was considered statistically significant.

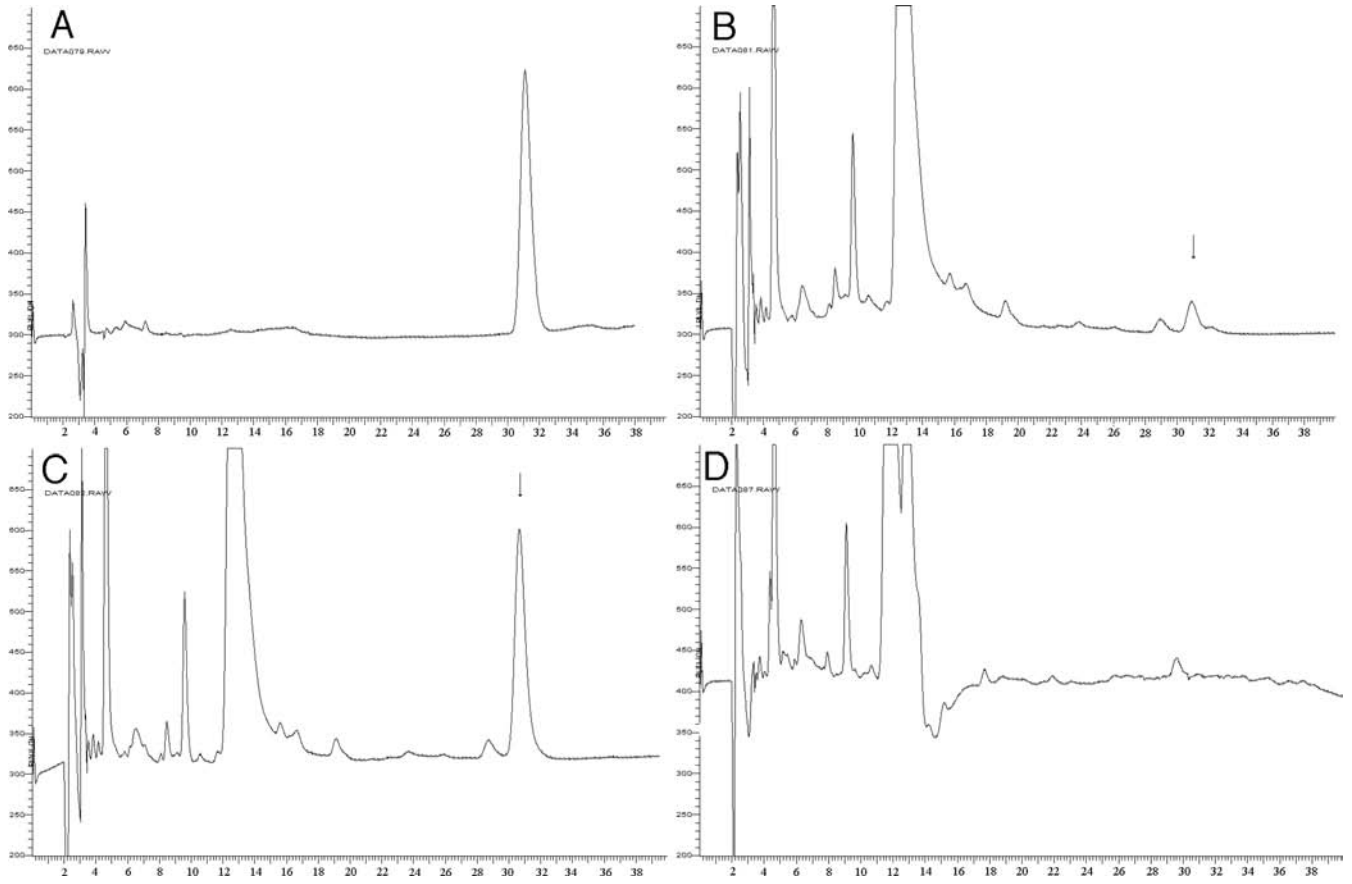

Figure 2. HPLC of (A) standard AECK-DD, (B) plasma sample from mouse fed AECK-DD-supplemented diet (0.05% w/w) for 1 day, (C) sample as in B added with standard AECK-DD, and (D) plasma sample from control mouse fed a basal diet. *X*-axis, time (min); *Y*-axis, detector response (mV). The plasma extraction procedure and HPLC conditions were as reported in the Materials and Methods.

RESULTS

Identification of AECK-DD in Plasma Samples by HPLC-ECD Analyses. The chromatogram obtained in HPLC-ECD of standard AECK-DD, eluting at 31.0 min, is reported in Figure 2A. Figure 2B shows a typical liquid chromatogram obtained from plasma of mice fed the AECK-DD-supplemented diet (0.05% w/w) for 24 h. A peak was identified as AECK-DD on the basis of both retention time and coelution with standard AECK-DD (Figure 2C). Noteworthy, AECK-DD was almost undetectable in the plasma of control mice (Figure 2D) fed the basal diet (without AECK-DD supplementation), according to the fact that only a very low amount of AECK-DD was detected in the control diet (10.1 ± 1.2 ng/g diet).

Identification of AECK-DD in Plasma Samples by GC-MS Analyses. The absorption of AECK-DD from diet in mice was also confirmed by GC-MS analyses, where the mass spectrometer was used in both scan and SIM modes. In Figure 3A, a typical GC-SIM-MS chromatographic profile of a plasma sample from mice fed AECK-DD supplemented-diet (0.05% w/w) for 24 h is reported, showing a peak having the same retention time (13.074 min) of standard AECK-DD (Figure 3B) and coeluting with standard AECK-DD. Only traces of AECK-DD were present in plasma sample from control mice fed the control, unsupplemented diet (Figure 3C). The mass spectra of the standard AECK-DD and AECK-DD extracted from plasma sample from AECK-DD-supplemented mice are compared, respectively, in Figure 4A,B. Both fragmentation patterns show the presence of the molecular ion M^+ 228 and of other typical ions (*m*/*z* 200, *m*/*z* 154, *m*/*z* 126, *m*/*z* 99, and *m*/*z* 71) with similar relative intensities. Similar results were obtained from the analyses of liver homogenates (data not shown). In both plasma and liver samples from mice fed the AECK-DD-supplemented diet, GC-MS analysis revealed the presence of the oxidative dehydrogenation product of AECK-DD. In the chromatographic profile of plasma sample from mice fed AECK-DD supplemented-diet (Figure 3A) for 24 h, a peak at retention time 14.14 min shows the same fragmentation pattern (Figure 5A) of standard dehydro-AECK-DD (Figure 5B), with the molecular ion M^+ 226 and other typical ions (*m*/*z* 221 and *m*/*z* 180). The dehydrogenation product of AECK-DD is absent in the chromatogram of plasma from control mice. Again, similar results were obtained from the analyses of liver homogenates (data not shown).

Time and Dose Dependence of AECK-DD Absorption in Plasma and Liver. Plasma and liver concentrations of AECK-DD in 24 h supplemented mice were clearly dose-dependent (Table 1). The plasma AECK-DD concentration was 39.7 ± 6.0 and 126.0 ± 12.3 ng/mL in mice supplemented with AECK-DD at 0.05 and 0.2% in the diet, respectively, while AECK-DD was undetectable or present as traces in plasma of mice fed the basal control diet. The liver AECK-DD content was 150.5 ± 12.0 and 432.0 ± 45.0 in mice supplemented with AECK-DD at 0.05 and 0.2% in the diet, respectively. Again, AECK-DD was undetectable or present as traces in the livers of mice fed the basal control diet.

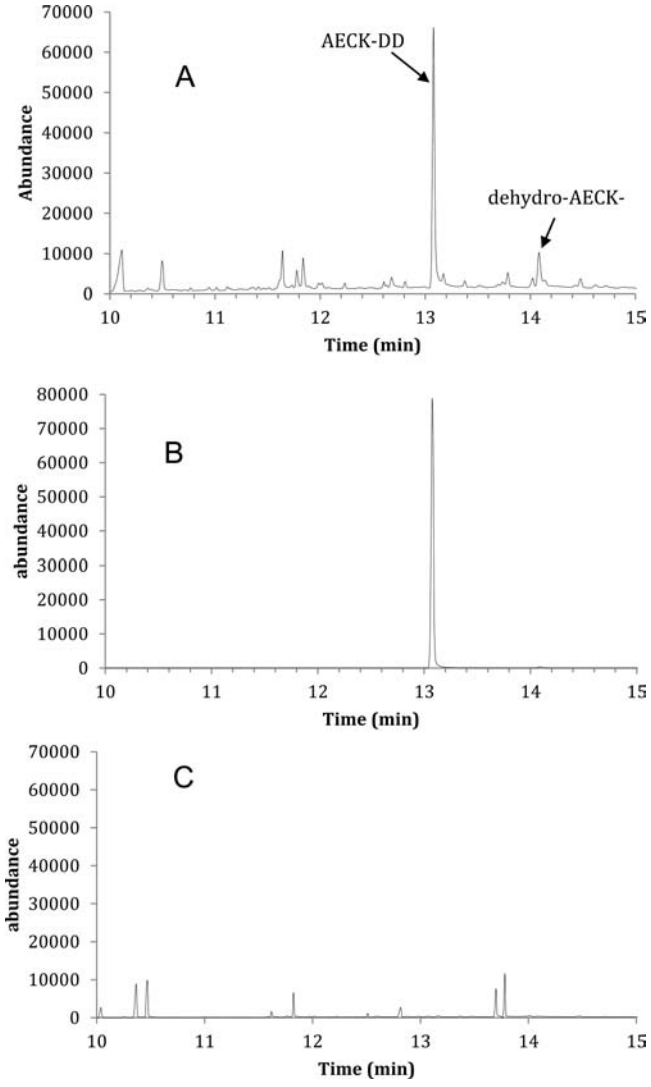

Figure 3. GC-SIM-MS chromatographic profile of plasma samples: (A) plasma from AECK-DD-fed mice, (B) standard AECK-DD, and (C) plasma from control mice.

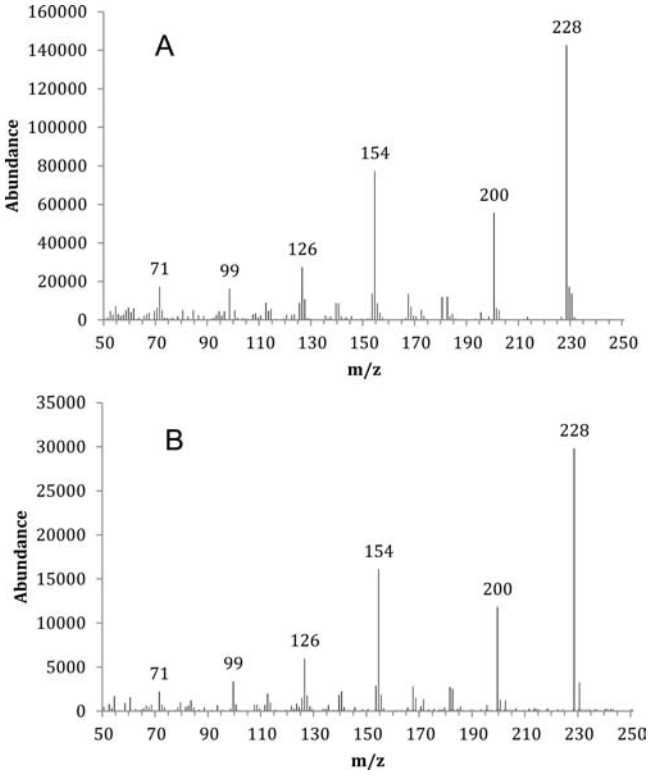

Figure 4. MS spectra of (A) standard AECK-DD and (B) plasma AECK-DD from AECK-DD-fed mice.

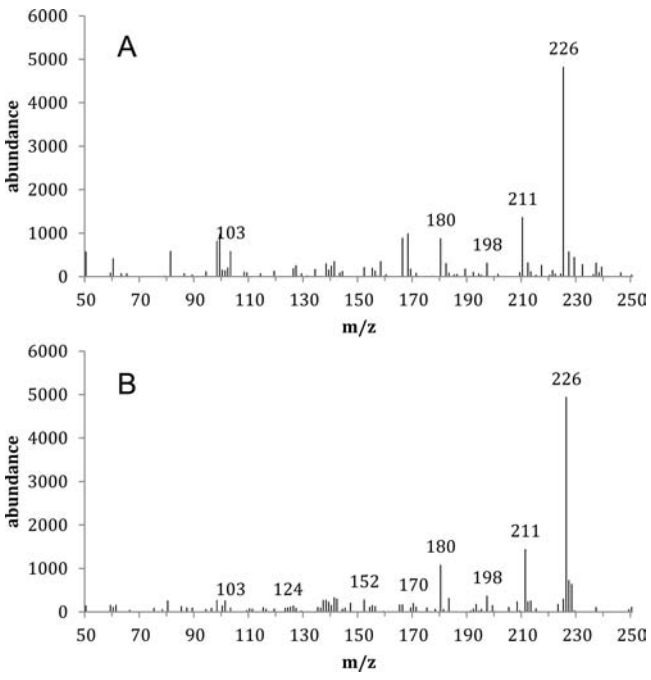

Figure 5. MS spectra of (A) plasma dehydro-AECK-DD from AECK-DD-fed mice and (B) standard dehydro-AECK-DD.

The effect of AECK-DD supplementation time on plasma and liver AECK-DD content and plasma antioxidant activity is shown in Table 2. Again, AECK-DD was undetectable or present as traces in plasma and liver of control, unsupplemented mice. Diet supplementation with 0.2% AECK-DD resulted in the appearance of detectable amounts of AECK-DD in both plasma and liver. The plasma AECK-DD content seems to increase with time, reaching a maximum at 5 days supplementation; however, no significant differences among the levels measured after 1, 5, and 10 days supplementation were found, suggesting that the plasma AECK-DD concentration reached a plateau. The liver AECK-DD content significantly increased with time, reaching a 3-fold increase at 10 days with respect to 1 day of supplementation. The levels of liver AECK-DD at 10 days of supplementation are higher than those measured after 5 days; however, this difference did not reach statistical significance.

Effect of AECK-DD Supplementation on Plasma Antioxidant Potential. In Figure 6A, the concentration–response curve of the percent inhibition of absorbance at 734 nm for $ABTS^{\bullet+}$ as a function of concentration of standard AECK-DD is shown, in comparison to that of trolox (Figure 6B), the water-soluble analogue of vitamin E. AECK-DD exhibited a strong antioxidant activity, in the same order of trolox, as demonstrated by very similar slope values (slope, 2.3802, for AECK-DD, r = 0.999; 2.4055 for trolox, r = 0.998).

The plasma antioxidant activity measured by ABTS assay was raised significantly following AECK-DD supplementation, with 8.0, 14.9, and 13.7% increases of the percent inhibition of

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Table 1. Effect of AECK-DD Supplementation Dose on Plasma and Liver AECK-DD Contents[a]

		AECK-DD in the diet	
	control	+0.05%	+0.2%
AECK-DD ng/mL plasma	tr[b]	39.7 ± 6.0 a	126.0 ± 12.3 b
AECK-DD ng/g liver	tr[c]	150.5 ± 12.0 a	432.0 ± 45.0 b

[a]Plasma and liver samples from mice supplemented for 24 h with 0 (control), 0.05, or 0.2% AECK-DD were analyzed for AECK-DD content by HPLC-ECD as reported in the Materials and Methods. Values with different letters were statistically different by one-factor ANOVA (Fisher method) ($p < 0.01$, $n = 4$); tr, traces. [b]AECK-DD ≤ 10.0 ng/mL plasma. [c]AECK-DD ≤ 4.0 ng/g liver.

absorbance at 734 nm at 1, 5, and 10 days of supplementation, respectively, in comparison to mice fed the control diet (Table 2). As observed also for the plasma AECK-DD content, the maximum effect was observed at 5 days of supplementation. Similar results were obtained using the ferric-reducing antioxidant power (FRAP) assay[22] (data not shown).

DISCUSSION

The first finding of this study is that exogeneous AECK-DD from AECK-DD-supplemented diet is absorbed in mice, in a dose- and time-dependent manner. Micromolar plasma concentrations of AECK-DD (range, 0.2−1.1 μM) were detected in AECK-DD-supplemented mice. AECK-DD was also incorporated in liver tissues. Interestingly, the dehydrogenation product of AECK-DD was detected in both plasma and liver of AECK-DD-fed mice, while it was undetectable in mice fed the control diet.

The second finding is that AECK-DD supplementation of mice results in a significant increase of plasma antioxidant potential, measured by ABTS assay. Moreover, the antioxidant activity of standard AECK-DD measured by ABTS method was found to be quite strong, in the same order of that of trolox, the water-soluble analogue of vitamin E.

Finally, only traces of AECK-DD were detected in plasma and liver samples of control mice, according to the fact that the control diet contains only very low amounts of AECK-DD. These results might suggest the absence of a biosynthetic pathways leading to endogeneous AECK-DD production in mice.

Our results demonstrate for the first time an antioxidant action of AECK-DD in ex vivo experiments. The concentration of AECK-DD measured in plasma of AECK-DD-fed mice (range, 0.2−1.1 μM) in this study is in the same order of those measured in human plasma from healthy subjects (range, 0.5−3.3 μM).[9,12] Therefore, from these results, it can be suggested that, at the concentrations present in human plasma, AECK-DD might really play a significant role in the modulation of plasma antioxidant potential and of oxidative process in vivo. The detection of the dehydrogenation product of AECK-DD further supports the antioxidant role of AECK-DD in vivo. In fact, dehydro-AECK-DD arises from oxidative dehydrogenation of AECK-DD, and it has been identified in in vitro oxidation experiments of AECK-DD with $CuCl_2$ and *t*-butyl hydroperoxide or with 2,2′-azo-bis-2-amidinopropane hydrochloride (ABAP).[19] The presence of dehydro-AECK-DD in plasma and liver of AECK-DD-fed mice further demonstrate that AECK-DD is absorbed and metabolized in mice. Moreover, from our results, AECK-DD was found to be a strong antioxidant, as efficient as trolox, the water-soluble analogue of vitamin E. This finding is in agreement with our previous results obtained with

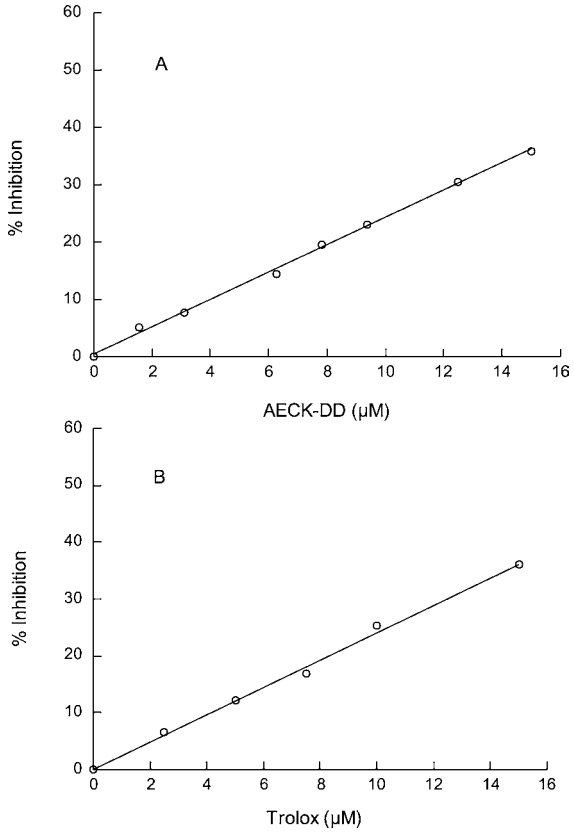

Figure 6. Concentration−response curve for AECK-DD (A) and Trolox (B) in the ABTS assay. The percentage inhibition of absorbance at 734 nm is calculated and plotted as a function of the concentration of the antioxidant tested (AECK-DD; $r^2 = 0.999$; slope, 2.3802. Trolox: $r^2 = 0.998$; slope, 2.4055). Values are means of two independent experiments, each performed in triplicate. Student's *t* test was used for correlation analyses.

Table 2. Effect of AECK-DD Supplementation Time on Plasma and Liver AECK-DD Contents and Plasma Antioxidant Activity[a]

	control	1 day	5 days	10 days
AECK-DD ng/mL plasma	tr[b]	126.0 ± 33.4 a	210.5 ± 52.7 a	150.6 ± 62.2 a
AECK-DD μg/g liver	tr[c]	0.4 ± 0.1 a	1.1 ± 0.3 b	1.6 ± 0.5 b
ABTS % inhibition	40.2 ± 1.5 a	43.4 ± 1.3 ab	46.2 ± 0.9 b	45.7 ± 0.9 b

[a]Plasma and liver samples from mice supplemented for the specified time with 0.2% AECK-DD were analyzed for AECK-DD contents by HPLC-ECD as reported in the Materials and Methods. The plasma antioxidant potential was measured on plasma samples by ABTS assay as described in the Materials and Methods. Values with different letters were statistically different by one-factor ANOVA (Fisher method) ($p < 0.05$, $n = 5$); tr, traces. [b]AECK-DD ≤ 10.0 ng/mL plasma. [c]AECK-DD ≤ 4.0 ng/g liver.

human monocytic U937 cells under hydroperoxide-induced oxidative stress.[15]

Oxidative stress is involved in many pathologies affecting human beings, such as atherosclerosis, cancer, neurodegenerative diseases, and aging.[23,24] Antioxidants counteract oxidative stress and slow down or prevent oxidative damage, with beneficial effects on human health. Because of its very low solubility in water, AECK-DD can be assumed to be bound to hydrophobic regions of macromolar constituents and to protect lipids from oxidation. In this regard, AECK-DD has been described to be associated to plasma lipoproteins in humans and to protect human low-density lipoproteins from oxidation in ex vivo experiments.[14] Moreover, an antioxidant action of AECK-DD inside the cells and its ability to modulate cellular response to oxidative challenge also have been demonstrated.[15] Interestingly, both the protective effects of AECK-DD against low-density lipoproteins oxidation and the cellular oxidative stress are elicited at micromolar concentrations (0.5−5 μM), close to those found in human plasma[15] and in plasma of AECK-DD-supplemented mice in the present study.

Within the past several years, AECK-DD was detected in many mammalian tissues and fluids.[9−11,13] However, a biochemical route leading to the in vivo synthesis of this compound has never been demonstrated in mammals. One of the current hypotheses is that AECK-DD can be formed in vivo from cysteamine and serine by the action of cystathionine β-synthase and glutamine transaminase followed by dimerization and decarboxylation reaction.[8,25] Recently, Pinto and co-workers reported that the brain of rat seems to have the capacity to synthesize AECK-DD when supplied with cysteamine (250 mg/kg body weight) by gavage treatment.[13] However, they were unable to detect AECK-DD in the brain of adult rats fed a standard rat chow, without cysteamine supplementation.[13] In the present study, we found only trace amounts of AECK-DD in plasma and liver of mice fed the control diet. These traces of AECK-DD found in plasma and liver of control mice could come from diet, due to the fact that the control diet contains a very low amount of AECK-DD, likely due to its maize wheat and soya derivatives contents. Recently, AECK-DD has been identified in some common vegetables present daily in the mediterranean diet such as garlic, onion, courgette, pepper, asparagus, spinaci, tomato, and aubergine.[12] The content of AECK-DD in these vegetables is lower with respect to that of the lipophilic antioxidant α-tocopherol, the most abundant form of vitamin E, as evaluated by literature data.[26,27] Accordingly, the plasma AECK-DD concentration in humans has been reported to be in the range 0.5−3.3 μM,[9,12] which is about 10-fold lower on average with respect to the plasma vitamin E concentration.[9,12,28,29] Nevertheless, from our results, plasma levels of AECK-DD in the order of micromolar concentration (similar to those measured in human plasma of healthy humans) significantly affect the total antioxidant potential of plasma.

The occurrence of AECK-DD in common vegetables of the human diet,[12] the presence of this molecule in human plasma at micromolar concentrations, and the lack of a demonstrated biosynthetic pathway in humans up to now might account for a dietary supply of this molecule. Our results indicate that exogeneous AECK-DD present in the diet is absorbed in mice, incorporated in liver, circulates in the plasma at micromolar concentrations, and significantly contributes to the total plasma antioxidant activity. Further studies are needed to investigate AECK-DD contents of a wide variety of foods and to evaluate the supply of AECK-DD from the diet.

AUTHOR INFORMATION

Corresponding Author

*Tel: +39 06 51494454. Fax: +39 06 51494550. E-mail: nardini@inran.it.

Notes

The authors declare no competing financial interest.

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