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

We wish to comment on a recent paper in this *Journal* by Piazzon et al.[1] These authors supplemented the diet of experimental mice with 0.05 or 0.2% (w/w) aminoethylcysteine ketimine decarboxylated dimer (AECK-DD; systematic name 1,2-3,4-5,6-7,8-octahydro-1,8a-diaza-4,6-dithiafluoren-9(8a*H*)-one; Figure 1A) and noted the appearance of AECK-DD in the plasma and liver in a time- and dose-dependent manner. Piazzon et al.[1] reported plasma levels of AECK-DD of 39.7 ± 6.0 ng/mL (0.17 ± 0.03 μmol/L) and 126.0 ± 12.3 ng/mL (0.55 ± 0.05 μmol/L) in the mice supplemented with 0.05 and 0.2% AECK-DD in the diet for 24 h, respectively. These dosages correspond to 3.5 mg (15.4 μmol) and 14.0 mg (61.4 μmol) of AECK-DD per day and per mouse, respectively. The levels of hepatic AECK-DD in these animals were reported to be 150.5 ± 12.0 and 432.0 ± 45.0 ng/g liver, respectively,[1] which translates to approximately 0.83 and 2.4 μmol/L, respectively, assuming a water content of about 80%. Previous work has shown that AECK-DD is an antioxidant, comparable to α-tocopherol and even more potent than ascorbate and glutathione.[2] The compound resulting from oxidation (dehydrogenation) of AECK-DD (named dehydro-AECK-DD by Piazzon et al.[1]) is shown in Figure 1B. Interestingly, Piazzon et al.[1] were able to detect this compound in the plasma of mice fed 0.05% AECK-DD in the diet for 24 h. The concentration of dehydro-AECK-DD was not specified, but from a comparison of the intensity of the AECK-DD peak to that of the dehydro-AECK-DD peak in the GC-SIM-MS chromatographic profile shown by the authors (Figure 3 in ref 1), we estimate that the concentration of dehydro-AECKK-DD in the plasma was about 10% that of AECK-DD. The authors also reported a significant increase in the plasma antioxidant activity in the mice fed AECK-DD in the diet compared to that observed in the plasma of mice fed standard laboratory chow.[1] The authors conclude in the last sentence of their abstract that "These results demonstrate for the first time the absorption of AECK-DD from [the] diet and the physiological relevance of this compound through its antioxidant action in vivo."[1] However, we have several reservations concerning the physiological relevance of the results reported by Piazzon et al.[1]

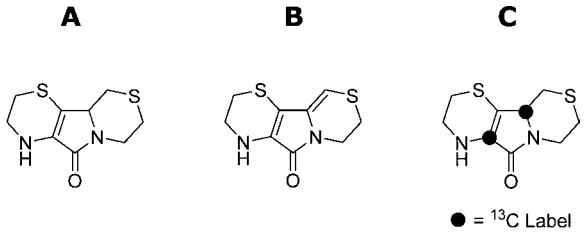

Figure 1. Chemical structures of AECK-DD (A), dehydro-AECK-DD (B), and $^{13}C_2$-AECK-DD (C).

As discussed by Piazzon et al.,[1] AECK-DD has been reported to be present at micromoles per liter concentrations in normal human plasma,[3] normal human urine[4] and bovine cerebellum.[5] Here, we wish to compare these published results with our previous findings. We recently reported a highly specific and sensitive GC-MS/MS approach for the reliable quantitation of AECK-DD in plasma, urine, tissue, and dietary vegetables using a newly synthesized and structurally fully characterized ^{13}C-labeled AECK-DD ($^{13}C_2$-AECK-DD) as internal standard (Figure 1C).[6] We performed quantitative analyses in the selected-reaction monitoring (SRM) mode using electron-capture negative-ion chemical ionization (ECNICI) and several different *m*/*z* transitions for physiological and $^{13}C_2$-AECK-DD, including those for the stable ^{34}S isotope (natural abundance, 4.22%). In our GC-MS/MS method,[6] SRM ensures high selectivity, ECNICI provides maximum sensitivity, and the use of $^{13}C_2$-AECK-DD as internal standard enables accurate quantification of AECK-DD, in contrast to the GC-MS method described by Piazzon et al.,[1] which uses less specific SIM, considerably less sensitive electron ionization (EI), and no labeled internal standard for quantification.[3,4] Using the GC-MS/MS approach, we found that the level of AECK-DD in body fluids is lower than that reported previously by others.[3,4] Thus, we found that AECK-DD is not present in plasma of healthy humans at concentrations above 4 nmol/L.[6] Interestingly, Piazzon et al.[1] reported AECK-DD plasma concentrations of ≤10 ng/mL (≤43.9 nmol/L) in nonsupplemented mice (control group). We noted that urine also has very low AECKK-DD concentrations.[6] However, we did show that AECK-DD is present at a maximal concentration of 46 nmol/L in urine samples obtained from the same cohort of human volunteers from which we obtained the plasma samples.[6] This value, although considerably lower than that reported previously for human urine, may nevertheless suggest that, in humans, AECK-DD accumulates in urine.

How do our values for rodent brain AECK-DD compare to those published for bovine cerebellum? In previous work, we used HPLC with CoulArray detection to measure AECK-DD in rat brain.[7] We reported that AECK-DD reached a concentration of about 40 pmol/mg of protein after administration of pharmacological doses of cysteamine to rats.[7] In our more recent study, using the more sensitive GC-MS/MS approach, we found a very low level of AECK-DD in normal (i.e., untreated) rat brain (~8 pmol/g wet weight; ~10 nmol/L).[6] Thus, the level of AECK-DD in normal rat brain is far lower than that reported previously for bovine brain.[5] However, there appears to be considerable species variability because, in the same study, AECK-DD was detectable in mouse brain at a content of ~1 nmol/g wet weight (~1.2 μmol/L).[6] Previous

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work has suggested that AECK-DD is generated from serine and cysteine by the action of cystathionine β-synthase.[7,8] Our findings of increased brain AECK-DD in rats treated with high doses of cysteamine is consistent with this hypothesis.[7] Moreover, mice deficient in transporters for cysteamine have less cerebral AECK-DD than control littermates.[6]

Taken together, our studies demonstrate that AECK-DD is indeed a natural product, but its concentration in rodent brain and human body fluids is generally extremely low. Nevertheless, because commercial rodent chow is very low in AECK-DD,[1,6] our findings do not preclude the possibility that AECK-DD will be at a higher concentration in brain (and other tissues) if the diet is rich in AECK-DD. Previous work has reported that of six common dietary vegetables, AECK-DD is present at levels ranging from 80 nmol/kg (aubergine) to 680 nmol/kg (onion).[9] Thus, Piazzon et al.[1] conclude that a diet rich in vegetables containing high levels of AECK-DD may result in body tissues/fluids containing concentrations of AECK-DD that provide considerable antioxidant defense. However, as we have already noted, our values for human plasma AECK-DD are much lower than those reported previously in the literature. Moreover, using our GC-MS/MS method, we have not been able to reproduce the findings of widespread occurrence of AECK-DD in vegetables.[6] Thus, of nine fresh dietary vegetables obtained from a local market (carrot, courgette, cucumber, endive, garlic, napa cabbage, pepper, romaine lettuce, shallot), AECK-DD could be detected in only shallots and, even in this vegetable, the AECK-DD content was about 6.8 nmol/kg wet weight, that is, at least 10−100 times lower than the levels reported by others to be generally present in a variety of vegetables.[9] To illustrate this point, we report here a GC-MS/MS chromatogram from the analysis of AECK-DD in which no AECK-DD was measurable above 5 nmol/kg fresh tissue (Figure 2).

In the study by Piazzon et al.,[1] AECK-DD was administered to mice at an average dose of about 660 μmol/kg body weight in the 0.05% group and 2640 μmol/kg body weight in the 0.2% group. Even on the assumption of an average AECK-DD content of 680 nmol/kg vegetable,[9] translation of the dosage used in mice in the study by Piazzon et al.[1] to humans would mean that subjects weighing 70 kg would need to consume daily about 68−272 kg fresh vegetables to increase the plasma antioxidant activity by a thousandth part of the increase measured in mice by the ABTS assay applied in the study by Piazzon et al.[1] Although this can be only a very rough estimate and the ABTS assay is compromised by several flaws and pitfalls,[10] we do have reservations concerning the relevance of AECK-DD as a physiologically relevant antioxidant in dietary vegetables.

In conclusion, AECK-DD occurs naturally, probably at least in part from the metabolism of cysteamine. Our analyses, based on the use of GC-MS/MS in combination with the use of a stable-isotope labeled AECK-DD, suggest that its presence in human body fluids and vegetables is extremely low (orders of magnitude lower than previous reports). Thus, although AECK-DD may prove to be a useful antioxidant in an experimental setting, its biological and dietary importance in a normal physiological setting may be less than previously suggested. One possible exception is the finding of AECK-DD in mouse brain at a level of ~1 μmol/L. The origin and importance of AECK-DD in mouse brain warrant further study.

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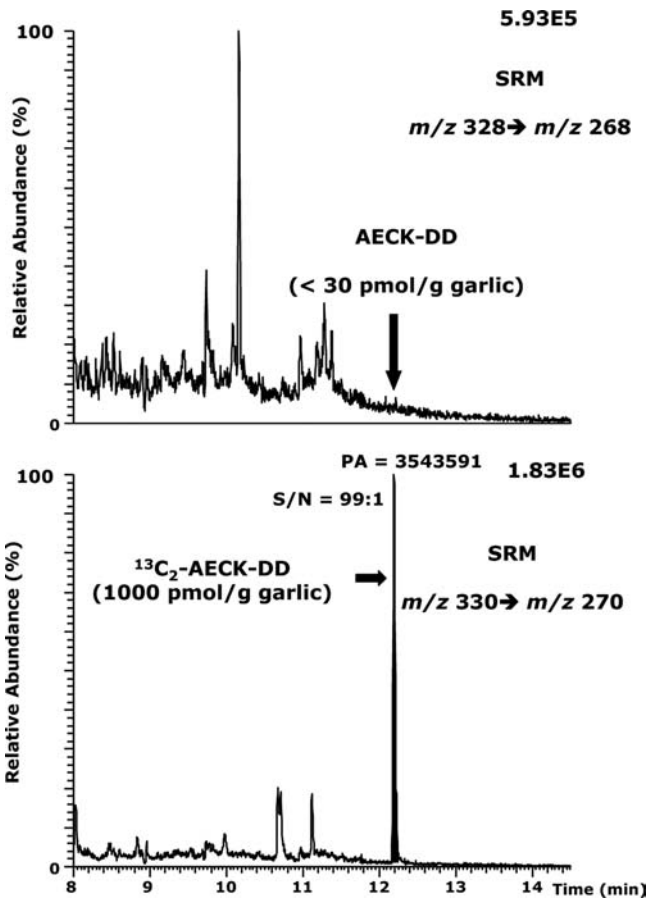

Figure 2. Chromatogram from the GC-MS/MS analysis of the pentafluorobenzyl (PFB) derivatives of natural AECK-DD (upper trace) in fresh garlic and synthetic $^{13}C_2$-AECK-DD serving as the internal standard (lower trace). $^{13}C_2$-AECK-DD was externally added to 1 mL of ice-cold 100 mmol/L phosphate-buffered saline (pH 7.0) that was used to homogenize garlic (1 g), resulting in a final content of 1000 pmol of $^{13}C_2$-AECK-DD/g fresh tissue. Quantification was performed by SRM of the transitions m/z 328 → 268 for AECK-DD and m/z 330 → 270 for $^{13}C_2$-AECK-DD. The complete GC-MS/MS approach has been described in detail elsewhere.[6] The PFB derivative of $^{13}C_2$-AECK-DD eluted at 12.19 min. The arrow in the upper panel indicates the retention time of the PFB derivative of natural AECK-DD. By using the signal-to-noise (S/N) ratio of $^{13}C_2$-AECK-DD and by approximating to a S/N value of 3:1, the AECK-DD content in the garlic sample analyzed is estimated to be <30 pmol/g garlic. This value is of the same order of the AECK-DD content of fresh shallot.[6] PA, peak area.

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Notes

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