The Role of Acetate in the Antagonization of Oxalate: A Potential Causative Molecule for Heart Disease and Cancer Death

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Abstract

Previous reports demonstrated the anticancer effects and the protective effects on cardiovascular diseases of vinegar products. The molecular mechanism underlying these phenomena is not well elucidated. It was proposed that carcinogenesis is triggered by the formation of local strong acids such as HCl. Cancer cells may overproduce weak or moderate organic acids such as oxalate to antagonize strong acids, and calcium oxalate may cause organ failure and death. This study aimed at elucidating the underlying mechanism on the antagonism of apoptosis by acetate on oxalate. Quantitation of cell apoptosis of HEK293T cells in the presence of sodium oxalate and compounds with similar structures to oxalate was conducted by using Annexin V-fluorescein isothiocyanate/propidium iodide staining via flow cytometry. The data indicate that acetate could attenuate the proapoptotic functions of oxalate. This study yields insight into the anticancer and antidisease functions of vinegar products and opens up a new path in the use of weak acetic acid in the prevention and treatment of cancer.

Keywords

acetate, apoptosis, cancer-free, oxalate, vinegar

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It was reported that Chinese vinegar factories have had very few cancer cases in decades.1,4 The rates of cardiovascular diseases were also very low. Local buildup of hydrochloric acid (HCl) was proposed as one of the primary causative factors of most cancers.5,6 The inhabitants of the humid south coastal regions of China and Southeast Asia are at high risk of nasopharyngeal carcinoma, whereas those of non-coastal southern Yunnan Province in China have low nasopharyngeal cancer rates,7 suggesting that hydrogen bonding is involved in the carcinogenesis of the nasal cavity.8 It was recently postulated that weak acid counteracts strong acid such as HCl to reduce cancer risks.1,4 Calcium supplementation is capable of neutralizing HCl and reducing cancer incidences.5,6,9 Malignant cells may overproduce organic acids such as oxalate to antagonize strong acids5,10,11 and calcium oxalate causes cell senescence.3,4,10,11 (Figure 1). Similar in structure to oxalate, alcohol, and acetic acid are beneficial to heart disease patients and extend their lifespan,12,13 perhaps through the inhibition of oxalate generation. Glycolic acid, another compound structurally similar to oxalate, has been used extensively in skin-care products and reduces age-related wrinkles. Terminally ill cancer patients often develop heart disease.14 This study validates the idea that ethanol and acetate can counteract oxalate in apoptosis assays of human cells.

Molecules with similar structures to oxalate, such as ethanol, acetate, and alanine, attenuated the proapoptotic activities of sodium oxalate on HEK293T cells (Figure 2). The percentages of apoptotic and necrotic cells in HEK293T cells with or without oxalate were 28.39% and 2.48%, respectively. After co-treatments with oxalate and other molecules, the percentages of apoptotic and necrotic cells went down to 19.50%, 21.70%, and 21.24% with sodium oxalate plus...
alanine, sodium oxalate plus sodium acetate, and sodium oxalate plus ethanol treatments, respectively.

Many amino acid residues possess hydrogen bonding capacity. When they are adjacent to many positively charged basic amino acids in protein primary structure or tertiary structure, it triggers the gathering of protons and Cl⁻ and the local formation of HCl which is mutagenic and carcinogenic. The buildup of HCl in the cells induces a cellular defensive mechanism to counteract the proton stress. Once the cancer cells developed, it may cause overproduction of organic acid such as oxalate to maintain the favorable condition for the cancer cells to grow. Chemically, insoluble and rigid salts such as calcium oxalate antagonize strong acids, counteracting the harmful effects of HCl. Oxaloacetate is produced from the Krebs cycle and gluconeogenesis and subsequently it can be broken down to oxalate, enhancing the formation of calcium oxalate. The respiratory chain in cancer cells is compromised, resulting in the accumulation of oxalate via the shunt of the ongoing normal Krebs cycle. Valine and glycine collectively are over-represented in numerous causative factors of heart disease. The carbonyl bond lengths of these 2 amino acids

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**Figure 1.** Multiple organ failure is a manifestation of end-stage malignancies and cancer may be ameliorated with acetic acid.

**Figure 2.** Quantitation of cell apoptosis of HEK293T cells in the presence of sodium oxalate and compounds with similar structures to oxalate using Annexin V-fluorescein isothiocyanate/propidium iodide staining via flow cytometry. The percentages of normal (B3 quadrant), early apoptotic (B4 quadrant), late apoptotic (B2 quadrant), and necrotic (B1 quadrant) cells were measured 24 hours after treatments with 2 mM sodium oxalate and 2 mM compounds similar in structures to oxalate. (a-h) Control, sodium oxalate, alanine, sodium acetate, ethanol, sodium oxalate + alanine, sodium oxalate + sodium acetate, sodium oxalate + ethanol. $P = 0.000$, Pearson $\chi^2$ significances between oxalate and oxalate plus compounds with similar structures to oxalate, respectively (2-tailed, SPSS 22.0).
are longer than their counterparts in other amino acids and the weakened carbonyl bond gives rise to the cation affinity of carbonyl oxygen, particularly to divalent cations such as calcium. The buildup of oxalate in malignant cells results in the formation of insoluble and rigid calcium oxalate, leading to the appearance of complications such as heart disease. Corroborating this mechanism, heart disease sufferers usually manifest constipation problems.\textsuperscript{11,17,18} Similar in structure to oxalate, ethanol and acetic acid inhibit the generation or function of oxalate and elicited the antiapoptotic effects in the aforementioned assay. Alanine might inhibit the carboxylation of pyruvate to form oxaloacetate in gluconeogenesis. Alanine also shares limited structural similarity to that of oxalate and displayed antagonism against oxalate via the effects of the above-mentioned factors.

Calcium oxalate crystals were observed in the thyroid of 85.2\% of the individuals over 70 years of age and autopsied within 5 hours following death.\textsuperscript{19} Calcium oxalate crystals were also discovered in the heart of a patient who died of heart failure.\textsuperscript{20} The data presented in this study suggest that acetic acid and ethanol may be excellent substances to inhibit oxalate generation and to reduce mortality. It was demonstrated previously that the oxalate level was substantially higher in breast cancer tissue than adjacent normal breast tissue.\textsuperscript{21} Zhu et al found that intake of vinegar or 5\% acetate increased citrate and reduced calcium in urinary excretion and inhibited the renal formation of calcium oxalate crystals in a rat model.\textsuperscript{22} Zeng et al reported that the consumption of fermented vinegar was inversely associated with kidney stone formation.\textsuperscript{23} Renal stones are primarily calcium oxalate stones. Given that it is a weak acid and similar in structure to oxalate, acetic acid may prove a wonder molecule which counteracts both HCl and oxalate for the treatment of cancer.\textsuperscript{16} Despite that ethanol elicited a similar effect as acetate, alcohol overconsumption poses cancer risks given its hydrogen bonding capacity on the hydroxyl group.\textsuperscript{1,16,17}

**Experimental**

**Flow Cytometry**

The apoptosis and necrosis of cells were analyzed using the Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) staining Kit following the manufacturer’s instructions (KeyGEN, China). After incubation with the peptides for 24 hours in either the presence or absence of 2 mM sodium oxalate, the cells were digested using 0.25\% trypsin and then collected, followed by washing twice with fresh phosphate buffer saline. Binding buffer (300 \mu L) was added to resuspend the cells, which were then labeled with 5 \mu L Annexin V-FITC and 5 \mu L PI in the dark for 15 minutes. Fluorescence intensities were detected using a Beckman Coulter Gallios Flow Cytometer, with 525 nm excitation in FL1 for AnnexinV-FITC and 575 nm in FL2 for PI. Acquired data were analyzed with Kaluza software.

**Statistical Analyses**

All statistical analyses were performed using SPSS 22.0. The alpha level for all tests was 0.05. Analysis via Pearson $\chi^2$ distribution was conducted using the normal cell count and the combined apoptotic and necrotic cell count for flow cytometry experiments.

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