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学位論文題名	The combined administration of vitamin C and copper induces systemic oxidative stress and kidney injury. （ビタミンＣと銅による全身の酸化ストレス及び腎障害）
論文審査委員	委員長　教　授　大塚　稔久 委　員　教　授　中島　歩 委　員　准教授　北間　敏弘

学位論文内容の要旨

[Purpose]

Ascorbic acid (AA, also known as vitamin C) and Copper (Cu^{2+}) are well-known health supplements with many health-promoting actions. However, in combination, they produce several major oxidants, including superoxide, H_2O_2, and highly reactive hydroxyl radicals (OH•). Given that these oxidants have been critically involved in initiating and developing various diseases, we speculated that in vivo administration of AA plus Cu^{2+} could lead to systemic oxidative stress and organ injuries. This study was to address this possibility. Because the kidney is especially vulnerable to various toxicants, including ROS, the effect of AA and Cu^{2+} on the kidney has been the focus of this study.

[Methods]

C57BL6/J mice were administered various doses of AA and Cu^{2+}, either alone or in combination, via gavage for up to 3 wks. The changes in the systemic and local oxidative state were monitored through the level of protein thiol oxidation and free sulfhydryl groups (-SH). The changes in renal function, injury, and structure were assessed by detecting biochemical parameters (Blood Urea Nitrogen (BUN), creatinine, urinary proteins), injury markers (nephrin and podocin), and histopathology. In vitro, oxidative effects of AA plus Cu^{2+} were determined using rat renal tubular epithelial NRK-E52 cells. The generation of H_2O_2 by AA plus Cu^{2+} was measured with an assay kit.

[Results]

1. Administration of mice with 100 mg/kg AA and 1 mg/kg Cu^{2+}, alone or in combination, through gavage once per day for 5 to 21 days resulted in a marked elevation in protein oxidation and a concomitant reduction in -SH in serum, intestine, bladder and kidney, indicative of an occurrence of systemic oxidative stress.

2. Administration of AA plus Cu^{2+} also led to a significant reduction in renal size and renal function, as evidenced by increased BUN, creatinine, and urinary protein levels. In addition, they also induced glomerular and tubular cell injury. These effects of AA plus Cu^{2+} were time- and dose-dependent.

3. In vitro, AA and Cu^{2+} in combination caused oxidative renal cell injury, which was associated with increased cellular protein oxidation. In addition, direct incubation of AA with Cu^{2+} led to H_2O_2 generation in an AA- and Cu^{2+} concentration-dependent manner.

4. The above-mentioned in vivo and in vitro effects of AA and Cu^{2+} were only observed when they were used in combination. Furthermore, the effects could be entirely prevented by thiol antioxidant NAC.

[Discussion]

AA is the most extensively used supplement. As an antioxidant, it has many health-promoting actions. However, AA also has prooxidant properties, especially in the presence of transition metal iron and Cu^{2+}. It has been reported that AA plus Cu^{2+} caused cell injury and induced biological protein oxidation in vitro. Here, we demonstrated, for the first time, that in vivo administration of AA plus Cu^{2+} caused systemic oxidative stress and oxidative kidney injury.

In this study, we used protein sulfenic acid formation to indicate oxidative stress. As the product of the reaction between a protein thiol and hydrogen peroxide, the level of sulfenic acid should reflect the in vivo level of H_2O_2. Taking advantage of this indicator, we observed that administration of AA plus Cu^{2+} markedly increased -SOH formation in serum and several organs, indicating the existence of systemic oxidative stress.

The kidney is an organ responsible for the filtration, reabsorption, and excretion of chemicals. In addition, renal cells are also known to express both AA and Cu^{2+} transporters. These features made the kidney especially vulnerable to AA and Cu^{2+}. Indeed, combined administration of AA and Cu^{2+} caused severe renal damage. The underlying mechanisms could be multiple, including a direct toxic effect derived from the locally generated ROS and an indirect effect caused by the oxidized serum proteins. In support of this notion, AA plus Cu^{2+} or oxidized albumin induced renal cell oxidation and injury in vitro.

Ascorbic acid has pro- and antioxidative actions. Unexpectedly, we failed to detect a noticeable change in the number of -SH in serum and cellular proteins when AA was used alone. The reason for the lack of antioxidative activity in our system is unclear and needs to be further analyzed. As for the prooxidant action of AA, it appeared that it required the presence of the transition metal Cu^{2+}.

Our study could have significant implications. First, our study demonstrated that in vivo administration of AA and Cu^{2+} caused systemic oxidative stress and kidney injury through ROS-related mechanisms. This feature of AA and Cu^{2+}could be used to develop novel models of ROS-driven kidney injury, through which the role of ROS in the initiation and development of kidney diseases could be clearly defined. Second, our study indicates that ascorbic acid and Cu^{2+}, as health supplements, should not be used together.

[Conclusion]

In conclusion, the current study reveals, for the first time, that the combined administration of AA and Cu^{2+} resulted in systemic oxidative stress and oxidative kidney injury. This property of ascorbic acid and Cu^{2+} could be exploited to establish the currently unreported ROS-driven models of kidney diseases. In addition, the finding from this study provides a warning of the health danger of the concomitant use of AA and Cu^{2+}, either as supplements or therapeutic regimens.

論文審査結果の要旨

ビタミン C（アスコルビン酸；AA）と銅（Cu2+）は、多くの健康促進作用を持つサプリメントとしてよく使われている。しかし、これらを併用するとフェントン反応が起こり、反応性の高いヒドロキシラジカルが生成される。腎臓はフリーラジカルを含む多くの毒性物質に対して脆弱であることから、申請者らは、AA と Cu2+の生体内投与が酸化的腎障害を引き起こすのではないかと仮定した。そこで、マウスに AA と Cu2+を単独または併用して、1 日 1 回経口にて、様々な期間で投与した。そして、全身の酸化状態の変化、および腎臓の構造と機能を解析した。そして、AA と Cu2+の投与は、血清、腸、膀胱、腎臓のタンパク質酸化を上昇させ、スルフェン酸形成の増加と遊離スルフヒドリル基（-SH）レベルの低下させることを明らかにした。AA と Cu2+によって誘発された全身性の酸化ストレスは、血中尿素窒素（BUN）、クレアチニン、尿蛋白の増加、糸球体および尿細管細胞の傷害によって示されるように、腎機能と構造の著しい変容と関連していた。興味深いことに、AA と Cu2+のこれらの作用は、併用した場合にのみ観察され、チオール系抗酸化剤 NAC によって完全に防ぐことができた。さらに、培養腎尿細管上皮細胞を用いた解析では、AA と Cu2+は細胞タンパク質の酸化と細胞死を引き起こし、NAC とカタラーゼによって抑制できることが明らかになった。さらに、AA と Cu2+の同時摂取は、過酸化水素の産生を引き起こした。以上の結果から、AA と Cu2+の併用投与は、全身性の酸化ストレスと腎細胞傷害を引き起こすことが明らかになった。申請者は、健康増進のためのサプリメントとして、AA と Cu2+は併用すべきではないと結論づけている。

これらの成果はすでに査読付きの国際誌に掲載済みである。詳細な分子メカニズムは不明ではあるものの、サプリメントに関する話題は社会的にも注目度が高く、タイムリーなテーマでもあった。得られた成果が実際にヒトにおいてどの程度影響があるのか、疫学的な解析を含めて今後詳細なデータが得られれば、さらなる発展が期待できる。