Inhibitory effect of catechin-related compounds on renin activity

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ABSTRACT
Renin is a crucial enzyme in the renin-angiotensin system, and the inhibition of its activity is considered as a useful approach to the treatment of hypertension. The inhibitory effect of catechin-related compounds on renin was investigated in this work. It was found that epigallocatechin gallate (EGCg) possessed the strongest activity with an IC50 value of 44.53 μM and acted in an uncompetitive manner. Gallated catechins exerted higher inhibition than the ungallated forms, and gallic acid exhibited an inhibitory potency close to that of epicatechin gallate (ECg). Results indicated that the galloyl moiety and ortho-trihydroxy phenyl structures might be favorable for the renin-inhibitory activity of these compounds.

Hypertension is significantly associated with the incidence of various cardiovascular diseases. The renin-angiotensin system (RAS) plays an important role in regulating blood pressure in animals, and its inhibition has been well established as an approach to the treatment of hypertension (15). In this system, renin (EC 3.4.23.15) and angiotensin I-converting enzyme (ACE; EC 3.4.15.1) are two key enzymes. Renin can cleave the N-terminus of angiotensinogen to yield angiotensin I, which is an inactive peptide and may further be processed by ACE to produce angiotensin II, a potent vasoconstrictor. Since the conversion catalyzed by renin is the rate-limiting step, it is thought that direct renin inhibition could lead to better suppression of high blood pressure than ACE inhibition can (2). Although many ACE inhibitors derived from foodstuffs have been identified in the past (7), only a few reports on natural renin inhibitors were available (6, 11, 20, 21). Studies have supported that polyphenol-containing materials such as black and green tea (14), red wine (12), cocoa extract (5, 17) and azuki bean seed coats (13) could reduce blood pressure in several experimental hypertensive models. The mechanisms underlying this effect were proposed to be attributable to vasodilator action, the attenuation of vascular oxidative stress and inflammation, and the ability to reduce arginase-2 activity (18), as well as the inhibitory effect against ACE (1, 3). Nevertheless, to date, not much attention has been paid to their potential renin-inhibitory activities. Recently, researchers found that saponins (8, 20, 21), baicalin (6), and the polyphenolic extracts of two green leafy vegetables (2) showed renin-inhibitory activities, which thus suggested the promising prospect of discovering polyphenolic compounds as candidates for renin inhibitors.

Catechins represent a major class of polyphenols and are widely distributed in plants. There are four principal kinds of catechins, that is, epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate; the beneficial effects of these compounds on health have been continually studied (4, 10). In the present work, we investigated whether catechin-related compounds could exert renin-inhibitory activities in vitro and also primarily elucidated their structure-activity relationship.

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Renin-inhibitory activity (Inhibition\%) = 

\[ \left( \frac{FI(\text{control}) - FI(\text{sample})}{FI(\text{control})} \right) \times 100 \]

The analyses were performed at least in duplicate and the IC_{50} value was defined as the concentration of the inhibitor required to inhibit 50\% of the renin activity. The inhibition mode of the most active compound on renin was analyzed with different substrate concentrations of 2.5, 5, and 10 μM, in the absence and presence of the inhibitor. The pattern of renin inhibition was evaluated using Lineweaver-Burk plots.

The renin-inhibitory activities of catechin-related compounds, including five kinds of catechins and gallic acid, are presented in Table 1. The results showed that although C and EC were almost ineffective in inhibiting renin activity, ECg, EGC, and EGCg exhibited inhibitory activities against renin, as follows: Renin-inhibitory activity (Inhibition\%) = 

\[ \left( \frac{FI(\text{control}) - FI(\text{sample})}{FI(\text{control})} \right) \times 100 \]

Table 1: IC_{50} values of catechin-related compounds against renin activity

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC_{50} (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>ND</td>
</tr>
<tr>
<td>EC</td>
<td>ND</td>
</tr>
<tr>
<td>ECg</td>
<td>619.40 ± 3.15</td>
</tr>
<tr>
<td>EGC</td>
<td>2175.30 ± 6.39</td>
</tr>
<tr>
<td>EGCg</td>
<td>44.53 ± 0.67</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>890.71 ± 1.63</td>
</tr>
<tr>
<td>Renin peptide inhibitor\a</td>
<td>0.17 ± 0.01</td>
</tr>
</tbody>
</table>

Results were the means ± SD of three analyses.

\a Z-Arg-Arg-Pro-Phe-His-Sta-Ile-His-Lys(Boc)-OMe, a synthetic specific renin peptide inhibitor served as control in this study.

ND indicated almost no renin inhibitory activity detected.

The catechin kit, containing (+)-catechin (C, Lot No. 081203), (−)-epicatechin (EC, Lot No. 090427), (−)-epicatechin gallate (ECg, Lot No. 090325), (−)-epigallocatechin (EGC, Lot No. 081117) and (−)-epigallocatechin gallate (EGCg, Lot No. 090427), with a purity over 98\% for each compound, was bought from Mitsui Norin Co. (Fujieda, Japan). Gallic acid (Lot No. 100M0258V) with a purity over 97\% was obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Fig. 1 depicts the chemical structures of these compounds. Z-Arg-Arg-Pro-Phe-His-Sta-Ile-His-Lys(Boc)-OMe, a specific renin peptide inhibitor served as control, was bought from Bachem AG (Bubendorf, Switzerland). The human recombinant renin inhibitor screening assay kit, which contained human recombinant renin, substrate (Arg-Glu(EDANS)-Ile-His-Pro-His-Leu-Ile-His-Thr-Lys(Dabcyl)-Arg) and Tris-HCl buffer (50 mM, pH 8.0, containing 100 mM NaCl), was purchased from Cayman Chemical Co. (Ann Arbor, MI, USA) for the measurement. The synthetic fluorescence resonance energy transfer peptide utilized in this assay is the normal substrate for renin and has been linked to a fluorophore at one end and to a non-fluorescent chromophore at the other end. After the peptide is cleaved by renin, the product obtained is highly fluorescent and can be easily analyzed by recording the fluorescence intensity (FI) on a fluorescence plate reader (Powerscan\b HT; BioTek Instruments, Inc., Winooski, VT, USA), with an excitation wavelength of 360 nm and an emission wavelength of 528 nm. The renin-inhibitory activity was calculated as follows: Renin-inhibitory activity (Inhibition\%) = 

\[ \left( \frac{FI(\text{control}) - FI(\text{sample})}{FI(\text{control})} \right) \times 100 \]
Catechins inhibit renin activity

Especially, the most bioactive compound EGCg tended to act as an uncompetitive inhibitor against renin, with concentration-dependent decrease in both $V_{\text{max}}$ and $K_m$ values, as presented in Fig. 3. The $K_i$ value for renin inhibition by EGCg was 45.71 μM, which was especially with the lowest IC$_{50}$ value of 44.53 μM (i.e., 20.41 μg/mL) observed in EGCg. Gallic acid could also exert renin inhibition with an IC$_{50}$ value of 890.71 μM (i.e., 151.53 μg/mL), which was relatively close to the inhibitory activity of ECg with an IC$_{50}$ of 619.40 μM (i.e., 274.00 μg/mL). It was reported that soybean saponin could inhibit human renin with an IC$_{50}$ value of 59.9 μg/mL (8). Fatty acids recognized as renin inhibitors in rice had an IC$_{50}$ value range of 28.3–41.1 μM (22). It was also confirmed that baicalin exerted renin inhibition with an IC$_{50}$ value of 120.36 μM (6). Li and Aluko reported that the functional peptides Ile-Arg, Lys-Phe and Glu-Phe showed renin-inhibitory activities, though the potency was relatively moderate with IC$_{50}$ values of 9.20, 17.84 and 22.66 mM, respectively (11).

Referring to these literatures, whereas the results might be affected slightly by the individual experimental conditions like reagents used, our findings indicated that order of the renin inhibitory activity was EGCg > ECg > Gallic acid > EGC, which could support their potential blood pressure-lowering properties.

As depicted in Fig. 2, the plots of percentage inhibition versus inhibitor concentration showed that it was in a dose-dependent manner for ECg, EGC, EGCg and gallic acid to inhibit renin activity. Furthermore, the Lineweaver-Burk plots indicated that

![Fig. 2 Plots of percentage inhibition on renin activity versus concentration of ECg (▲), EGC (●), EGCg (◆), and gallic acid (■). The error bars indicated the standard deviation of duplicates.](image)

![Fig. 3 Lineweaver-Burk plots of the inhibition on renin activity by EGCg. Renin-inhibitory activities were determined in the absence (●) and presence of 103.41 (◆) or 34.47 μM (▲) of the inhibitor. X axis represented the reciprocal of the substrate concentration ([S]) and Y axis represented the reciprocal of the reaction rate (V) indicated by fluorescence intensity unit per second (FIU/s). The error bars indicated the standard deviation of duplicates.](image)
directly correlated to its IC$_{50}$ value. It was suggested that EGCg exerted the bioactivity by forming an enzyme-substrate-inhibitor complex, which might influence the conformation of the active site of renin and consequently result in the loss of its enzymatic activity. The limited studies that have been performed in this regard reported that an uncompetitive inhibition pattern against renin was observed for the polyphenolic extract of Gongronema latifolium leaves (2) and flaxseed protein hydrolysate (23), while oleic acid and linoleic acid exhibited their renin-inhibitory activities as competitive inhibitors (22).

It was found that gallated catechins, that is, ECg and EGCg, showed higher renin-inhibitory activities than the ungalloated forms, suggesting that esterification with gallic acid could lead to quite favorable influence on elevating the renin-inhibitory activities of EC and EGC. Previous work reported that catechins with a galloyl moiety presented higher activities in various cases than those without this moiety, for example, with respect to inhibiting fatty-acid synthase (25), suppressing postprandial hypertriglyceridermia (19) and modulating the intestinal digestion of dietary starch (24). In the present study, taking into consideration that gallic acid itself exerted good renin-inhibitory activity close to that of ECg, it could be speculated that the galloyl moiety might be a critical structure for the inhibitory activities of catechin-related compounds against renin. On the other hand, it was also noticeable that EGCg, which had an extra ortho-trihydroxy phenyl structure except for a galloyl moiety, showed much stronger activity than ECg and gallic acid; EGC, which had only one ortho-trihydroxy phenyl structure, exhibited moderate activity; almost no activity was observed in C and EC without the trihydroxy structure. These results provided additional proof that the vicinal trihydroxy phenyl structure might also contribute to affecting the reactive site of renin and was helpful for exerting the renin-inhibitory activities of catechins. It has been reported that, for the renin-inhibitory activity of saponins, it was essential to have a glucuronic acid residue present at the first inner position of the 3β-hydroxyl sugar chain (21). In another study, the structure-function relationships of fatty acids were investigated, and it was discovered that unsaturated fatty acids exhibited inhibitory activities on recombinant human renin, while such an effect was not detected in saturated fatty acids (22). All these results implied that the renin-inhibitory activities from different categories of compounds could be attributed to the presence of special functional structures. It is necessary to evaluate the renin-inhibitory activities of the analogues containing the galloyl moiety without the catechin skeleton as well as those containing an ortho-trihydroxy phenyl structure, which would be helpful to elucidate more sufficiently the structure-activity relationship of renin inhibitors.

The antihypertensive effect of catechins had been proven in previous studies. For instance, EGCg and EC were reported to be able to improve endothelial function and reduce blood pressure in hypertensive rats, and the mechanism was partially contributed to the stimulation of nitric oxide production (9, 16). With respect to the influence on the key enzymes of the RAS, it was found that EGC could depress the ACE activity better than C, EC, and gallic acid (1). To the best of our knowledge, the present study is the first time to investigate and confirm the renin-inhibitory activities of catechin-related compounds. Moreover, it is well known that catechins constitute the major class of bioactive substances in tea. The association between tea consumption and blood pressure reduction has also been positively verified in experimental models, and the effect was considered mainly due to the favorable modification of the expression and function of oxidant and antioxidant enzymes (26). Hence, the results in this study not only indicated an additional potential pathway for the role of catechins in lowering blood pressure, but also provided evidence supporting tea consumption as an approach to helping control hypertension. Besides, EGCg has been the focus of research about health promotion effects in recent years (10). The present results provided more information regarding its bioactive properties, and the occurrence of its renin inhibition in vivo needs to be further investigated.

In conclusion, this study demonstrated the renin-inhibitory activities of catechins as well as gallic acid, and the strongest activity was observed in EGCg, which had an IC$_{50}$ value of 44.53 μM and acted in an uncompetitive mode. Gallated catechins, i.e., ECg and EGCg, showed higher inhibition than ungalloated forms. The galloyl moiety and ortho-trihydroxy phenyl might be essential structures for these catechin-related compounds to exert renin inhibitory activities. Further work is now in progress to investigate the renin-inhibitory activities of catechin-rich materials like different kinds of tea and to evaluate the correlation between the activities of the materials and the distribution and concentrations of catechins.
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