Purification and characterization of angiotensin-converting enzyme (ACE) from sheep lung

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Abstract
Angiotensin-converting enzyme (ACE, EC 3.4.15.1) in the renin-angiotensin system regulates blood pressure by catalyzing angiotensin I to the vasoconstrictor angiotensin II. In this study, the ACE was purified and characterized from sheep lung. The kinetic properties of the ACE were designated. The inhibition effect of captopril, a specific ACE inhibitor, was determined. ACE was purified from sheep lung using the affinity chromatography method in one step. NHS-activated Sepharose 4 Fast Flow as column filler and lisinopril as a ligand in this method used. The molecular weight and purity of ACE were designated using the SDS-PAGE method. Optimum temperature and optimum pH were found for purified ACE. \( K_M \) and \( V_{max} \) values from Lineweaver–Burk charts determined. The inhibition type, \( IC_{50} \), and \( K_i \) values of captopril on purified ACE were identified. ACE was 6405-fold purified from sheep lung by affinity chromatography in one step and specific activity was 16871 EU/mg protein. The purity and molecular weight of ACE were found with SDS-PAGE and observed two bands at around 60 kDa and 70 kDa on the gel. Optimum temperature and optimum pH were designated for purified ACE. Optimum temperature and pH were found as 40 °C and pH 7.4, respectively. \( V_{max} \) and \( K_M \) values were calculated to be 35.59 (µmol/min).mL\(^{-1}\) and 0.18 mM, respectively. \( IC_{50} \) value of captopril was found as 0.51 nM. The inhibition type of captopril was determined as non-competitive from the Lineweaver–Burk graph and the \( K_i \) value was 0.39 nM. As a result, it was observed in this study that the ACE enzyme can be successfully purified from sheep lungs in one step. Also, it was determined that captopril, which is a specific ACE inhibitor, has a significant inhibitory effect with a very low \( IC_{50} \) value of 0.51 nM.

Keywords Angiotensin-converting enzyme (ACE) · Characterization · Purification

Introduction
Hypertension is one of the most important health problems leading to coronary heart disease, myocardial infarction, and kidney disease [1]. Angiotensin-converting enzyme (dipeptidyl carboxypeptidase, EC 3.4.15.1; ACE) regulates blood pressure by converting the decapeptide angiotensin I to octapeptide angiotensin II, a potent vasoconstrictor, and inactivates the vasodilator bradykinin compound [2]. The enzyme hydrolyzes the angiotensin I, bradykinin, [3] but also the important hemoregulatory peptide, Acetyl-Ser-Asp-LysPro [4], as well angiotensin 1–7 [5] and substance P [6]. Also, the haemoregulatory peptide N-acetyl-Ser-Asp-Lys-Pro is a specific and natural substrate of the N-terminal active site of human ACE [4].

ACE is a zinc metallopeptidase that belongs to the dipeptidyl carboxypeptidase family. The human ACE enzyme has two functional N and C domains, each with an active site by a zinc ion binding site. Zinc is an important component in the catalytic binding site of ACE [7]. Helix 13 includes the canonical HEXXH zinc-connecting motif, using His 387 and His 383 along with Glu 411 on helix 14. The active site of ACE combines with several other stabilizing residues using the bound zinc [7]. The substrate connects the zinc by replacing it with the water compound connected to zinc. Following, the water compound combines with nearby Glu 384, causing polarization between the positive zinc ion and the negative glutamate carboxylate group [8]. This increases the nucleophilicity of water oxygen, triggering an assault on the peptide carbonyl carbon substrate. The proton taken up by the active site glutamate is sent to the nitrogen, presumably
forming a tetrahedral gem-diolate intermediate with the aid of Tyr 523. The dipeptide product protonated form consists of cleavage of the C-N bond. [7]. The residuary peptide substrate is balanced by hydrogen bond interplays between Ala 354 and the new terminal amide, His 513 and His 353 by the secondary carboxyl group, and Lys 511 and Tyr 520 and the terminal carboxylate [9].

The ACE enzyme is a high molecular weight integral membrane protein situated on the lumen surface of the cell membrane. There are two forms of ACE enzyme: the somatic form, which is abundant in the endothelial surface of the lung vessels, and a smaller germinal form found only in the testis [10, 11]. The somatic isoform has a molecular mass that varies from 130 to 180 kDa and was described in the endothelial [12], epithelial, mesangial, and neuronal cells [13, 14], and in the subsequent tissues as intestine, lung, kidney, pancreas, heart, placenta, and liver [15–17]. The germinal ACE of 90–100 kDa is similar to the C-terminal part of the endothelial ACE. Germinal ACE is limited solely to the testis found in germinal cells during the maturation of spermatogenesis [18]. Soluble ACE forms were described in urine and the ileal, seminal, and amniotic fluids [19–21], and plasma [22].

In the literature, N-domain ACE was described in ileal fluid and urine by Deddish et al. [19] and Casarini et al. [21, 23]. In urine, Casarini et al. [21, 23] observed two ACE isoforms by molecular weights of 65 and 190 kDa (N-domain ACE) in the urine of healthy subjects, and two isoforms of 65 and 90 kDa (both N-domain ACE) in the urine of patients with hypertension differing from the enzyme described by Deddish et al. also N-domain ACE from human ileum fluid with a molecular weight of 108 kDa [19]. Marques et al. found the same profile in the urine of Wistar-Kyoto and spontaneously hypertensive rats as described for the urine of healthy and patients with hypertension, and suggested the 90/80 kDa ACE isoforms as a likely genetic marker of hypertension [24].

ACE interacts simultaneously with the RAS and the kalikrein-kinin system, separating the C-terminal dipeptide from Angiotensin I and bradykinin. ACE has been observed to play a very important role in the balance between the vasodilatory properties of bradykinin and the vasoconstrictor features of angiotensin II. An increase in ACE activity disrupts this delicate balance, activates the vasoconstrictor angiotensin II and reduces the vasodilator bradykinin. ACE inhibitors reduce high blood pressure by reducing angiotensin II formation and increasing the bradykinin compound. Thus, these inhibitors restore this balance in hypertensive patients [25–27].

The affinity chromatography method is very sensitive and reduces the purification steps to a single step. Several thousand folds of purification can be made using this highly selective method. Therefore, the affinity chromatography method is generally used in purification processes [28]. The ACE enzyme was purified with lisinopril—affinity chromatography from human plasma [29], pig kidney [30], adult houseflies (Musca domestica) [31], swine serum [32], and pig lung [33]. In this work, NHS-activated Sepharose 4 Fast Flow as column filler in affinity chromatography method utilized. Lisinopril, a specific ACE inhibitor, as ligand utilized.

In this work, the ACE enzyme was purified from sheep lung with affinity chromatography and characterized. Optimum temperature and optimum pH of the ACE were found. $K_M$ and $V_{max}$ values determined. Until now, the ACE enzyme has not been purified from sheep lung in the literature. IC$_{50}$ value, the inhibition type, and the $K_i$ value of captopril on purified ACE were found.

### Materials and methods

#### Materials

Sodium tetraborate (Na$_2$B$_4$O$_7$.10H$_2$O), HepesNa, Coomassie Brilliant Blue R-250, N-[3-(2-Furyl)acryloyl]-L-phenylalanyl-glycyl-glycine (FAPGG), lisinopril, and Coomassie Brilliant Blue G-250 were bought from Sigma-Aldrich. NHS-activated Sepharose 4 Fast Flow was obtained from GE Healthcare Life Sciences. Captopril was purchased from Alfa Aesar.

#### Obtain of the sheep lung

Healthy sheep lungs, which were slaughtered in the slaughterhouse, were brought to the laboratory. Approximately 20 g of tissue was cut from different areas of the lung. This section was cut into small cubic pieces with a scalpel. The disrupted lung was added to 50 mM Tris (pH 7.4) buffer. This mix was subjected to disintegration for 3 min with the help of a mixer. Meanwhile, ice was placed around the mixer to prevent it from heating. The homogenate obtained was applied for 3 min in an ultrasonic homogenizer for further disintegration. Then, the mixture in the beaker was placed in the centrifuge tubes and centrifuged in a cooled centrifuge device at 8500 × g at +4 °C for 1 h. This was done several times. After centrifugation, the liquid from the top of the tube was taken and stored in the freezer for use in the purification process.

#### Affinity chromatography method

NHS-activated Sepharose 4 Fast Flow (25 mL) in 100% isopropanol was activated using the procedure of the manufacturer. To prepare the column, first, the gel was washed with 1 mM cold HCl. Second, a coupling tampon (5 mM of Lisinopril, 0.2 M of NaHCO$_3$, and 0.5 M of NaCl) was added.
to the affinity gel. Completion of the reaction was left at 4 °C overnight. The gel was then kept in 0.1 M Tris–HCl (pH 8.5) for several hours to prevent nonreacted groups on the affinity gel. Following, the gel was washed with 0.1 M acetate tampón (pH4.5) and 0.1 M Tris–HCl tampón (pH8.5). This process was done three times. Then, the affinity gel was suspended with an equilibration tampón (20 mM of Tris and 0.3 M of NaCl, pH 8.0).

The suspended affinity gel was packaged in a column (1 x 10 cm) with the equilibration tampón (20 mM of Tris and 0.3 M of NaCl, pH 8.0). The flow rates for washing and equilibration were determined using a peristaltic pump to 40 mL/h. After, the sheep lung was loaded onto the NHS–activated Sepharose 4 Fast Flow affinity column, and the column was cleaned by the equilibrium tampón. Subsequently, cleaning of the gel was continued until the absorbance was 0.1 at 280 nm. Following, sodium borate tampón (50 mM of Na2B4O7·10H2O, pH 9.0) was given to the column. The elution was then taken as 2.5 mL fractions. The ACE activity in the fractions was determined spectrophotometrically at 345 nm. The activity-containing tubes were pooled. All of the procedures were done at 4 °C [34, 35].

ACE Activity determination

The ACE activity was measured spectrophotometrically as the decrease in the absorbance at 35 °C and 345 nm according to the method of Holmquist [36]. The assay cuvette contained 50 mM HepesNa tampón (10 μM ZnCl2, 0.3 M NaCl, pH 7.5) and 1 mM FAPGG. One unit of activity was identified as the quantity of ACE that produces a ΔA345/min of 1.0 [33, 36].

Protein determination

The protein concentrations of the purified fractions and the lung homogenate were designated using Coomassie Brilliant Blue G-250 dye solution at 595 nm with the Bradford procedure. In this method, bovine serum albumin was utilized as the standard protein solution. The standard chart was drawn with the values found [37].

Determination of purity and molecular weight of ACE with SDS–PAGE method

Purity and molecular weight purity of the ACE, SDS–PAGE were determined according to Laemmli’s method. The acrylamide concentrations of the separating and stacking gels were prepared as 10% and 4%, respectively. Also, both the separating and stacking gels were found to have 1% SDS. After the process of running the proteins in the electrophoresis process was finished, the gel was removed. The gel was stained for 2 h in 0.025% Coomassie Brilliant Blue R-250 dye solutions including 7% acetic acid, 40% methanol, and bidistilled water. The gel was taken from the dye solution. The gel was first washed in the first wash solution (10% acetic acid, 50% methanol, and 40% bidistilled water). It was then washed in the second wash solution (7% acetic acid, 5% methanol, and 88% bidistilled water). The photo of the protein bands on the gel obtained was taken [38].

Optimum temperature determination

The optimum temperature of ACE purified from sheep lung was found using a constant temperature circulation bath. HEPES buffer, ACE, and FAPGG substrate to be used for activity measurement were added into the cuvette, mixed with a vortex and the first absorbance was taken in the spectrophotometer. Then the sample temperature was left in the adjusted constant temperature circulation bath. At the end of thirty minutes, it was removed from the constant temperature circulation bath and the final absorbance was read in the spectrophotometer. Measurements were made between 20–60 °C at 5 °C intervals. To designate the optimum temperature, the ACE activity was assayed at 345 nm in 50 mM HEPES tampon at the distinct temperature intervals in the range from 20 to 60 °C. The temperature was performed with a Grant bath (model 6G).

Optimum pH determination

HEPES buffers with pH 6.8, 7.0, 7.2, 7.4, 7.6, 7.8, 8.0, 8.2 were prepared to determine the optimum pH of ACE purified from sheep lung. ACE activities were measured individually using buffers at this pH. The same method was used for activity measurement. For the optimum pH designation, the ACE activity was performed in 50 mM HEPES tampon over the pH ranges 6.8–8.2. The activity measurement was made at 0.2 unit intervals between pH values of 6.8–8.2.

In vitro inhibition effect of captopril on ACE activity

The inhibition effect of captopril on ACE purified from sheep lung was explored. For this, distinct concentrations of the active substances were joined to the measurement cuvette (100 μL enzyme solution, 50 mM HepesNa, 10 μM ZnCl2, 0.3 M NaCl, 1 mM FAPGG,) for determination of the concentration interval, and ACE activities were measured. Percentage activity vs active substances concentration was plotted. The IC50 value of captopril was found from the equations of the inhibition plots. To plot Lineweaver–Burk graph and designate the inhibition type, five distinct concentrations of FAPGG and three different concentrations for captopril were used [39].
Results and discussion

In the present work, ACE was purified and characterized from sheep lung. NHS-activated Sepharose 4 Fast Flow was utilized as a column filling substance in the affinity chromatography method (Fig. 1). It was purified 6405 fold in one-step ACE with affinity chromatography method and its specific activity was designated as 16871 EU/mg protein (Table 1). In a work, ACE was purified approximately 7000 times from human lung tissue [40]. In a work by Erickson et al. ACE was purified 4500 fold from rat intestinal mucosa after affinity chromatography and gel filtration with lisinopril-sepharose. The specific activity of the purified ACE enzyme was calculated as 65 U/mg protein with benzoyl-Gly-His-Leu used as a substrate [41]. The purification factor of ACE enzyme purified from adult house flies (M. domestica) by lisinopril-sepharose affinity column chromatography was calculated as 709 [31]. In another work, ACE was 1588-fold purified from canine testes by lisinopril—affinity chromatography [35]. ACE was 308-fold purified from pig lung and the specific activity was 37 U mg⁻¹ [42]. Compared to alike works, the high purification coefficient in our work proves a successful purification in one step.

Angiotensin-converting enzyme (ACE) in a membrane-bound form in epithelial or neuroepithelial cells, endothelial cells, heart, lung, brain, kidney, and testes; It is a diivalent dipeptidyl carboxyl metallopeptidase present in a soluble form in the blood, serum, plasma, seminal and lymph fluid, cerebrospinal fluid and many-body fluids [43, 44]. In most of the studies, chemicals such as detergents have been used to purify the membrane-bound ACE enzyme. For example; Triton X-100 was utilized to be a detergent to purify the ACE enzyme from human lung tissue [40]. Detergent and protease inhibitors were used when purifying the ACE enzyme in canine testicles [18]. Triton X-100 was used when purifying the ACE enzyme from the ostrich (Struthio camelus) lung [45]. In our work, sheep lung homogenate was prepared with tris buffer (pH 7.4). As a result, soluble forms of ACE pass into the solution medium while preparing the lung homogenate. Soluble forms of ACE from sheep lung homogenate were purified by affinity chromatography. Therefore, in this study, a successful purification was performed in one step without using detergents such as Nonidet P-40, Triton X-100, trypsin, and protease inhibitors. In this case, it provided us a great advantage in terms of both time and costs while purifying.

In the present work, ACE was purified from sheep lung using affinity chromatography, in which lisinopril used as ligand and NHS-activated Sepharose 4 Fast Flow utilized to be column packing substance. Sodium tetraborate (pH 9) tampon was used as elution tampon and prosperous purification was performed in a one-step method. In some studies, ACE was purified from rabbit lung, human plasma, pig kidney, and pig striatum with lisinopril-affinity chromatography by Sepharose CL-4B, column packing substance. Hepes (pH 7.5) was utilized to be the elution tampon and 10 μM lisinopril was joined to Hepes tampon. Dialysis was performed against EDTA (pH7.5) tampon for 14 days to separate the lisinopril in the eluates [29, 46]. In this work, dialysis was not required since lisinopril was not joined to the elution tampon. Therefore, significantly high ACE activity was found in elutions that supply important advantages in both costs of the process and time.

Studies have shown that there are two forms of eACE. There are two isoenzymes, the first is the somatic ACE (130–180 kDa) found in somatic tissues like the kidney and lung, and the second is the testicular ACE (90–110 kDa), which includes solely one catalytic domain, which is the same as the C-terminal domain of the somatic ACE [47, 48].

![Fig. 1 Purification of the ACE enzyme with NHS-activated Sepharose 4 Fast Flow affinity chromatography column](image)

### Table 1 Purification scheme of angiotensin-converting enzyme (ACE) purified from sheep lung

| Purification steps | Activity (EU/mL) | Total volume (mL) | Protein (mg/mL) | Total protein (mg) | Total activity (EU) | Specific activity (EU/mg) | Yield (%) | Purification factor |
|--------------------|------------------|------------------|-----------------|-------------------|---------------------|--------------------------|-----------|------------------|
| Sheep lung         | 64.475           | 50               | 24.477          | 1223.85           | 3223.75             | 2.634                    | 100       | 1.0              |
| NHS-activated Sepharose 4 fast flow chromatography | 65.120           | 15               | 0.00386         | 0.0579            | 976.8               | 16871                    | 30        | 6405             |

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For instance; the molecular weight of the ACE enzyme purified from adult house flies (M. domestica) was determined by SDS-PAGE to be 67 kDa [31]. The ACE enzyme was purified from frog ovaries with affinity chromatography by extraction with detergent and trypsin. The molecular weight of the ACE enzyme extracted with both detergent and trypsin was determined to be 150 kDa [49]. The molecular weight of the ACE enzyme purified from rat intestinal mucosa was found as 160 kDa by SDS-PAGE [23]. In a work conducted by El-Dorry et al., The molecular weight of the ACE enzyme purified from rabbit testes was designated as 100 kDa [50]. Casarini et al. [21] characterized isoforms of the ACE N-domain with 65 and 90 kDa. This group has publications on mesangial cells, urine, tissue from Wistar and SHR mice. Andrade et al. did a spectroscopic and structural analysis of somatic and N-domain ACE isoforms from mesangial cells detecting differences between these enzymes [51]. Another study the same group described shadases able to liberate 65 and 90 kDa ACE isoforms in mesangial cells [52].

Also, in our previous studies, the molecular weight of the ACE enzyme purified from human plasma was found to be 60 and 70 kDa [53, 54]. In our work, purity and molecular weight of the ACE enzyme purified from sheep lungs were designated by SDS-PAGE, and two bands, 60 kDa and 70 kDa, were observed on the gel (Figs. 2 and 3). Since the ACE enzyme has two isoenzymes, distinct molecular weights were determined in distinct tissues that were purified.

Biochemical parameters were found to further characterize the ACE enzyme purified from sheep lung. Measurements were made for the purified ACE enzyme at 5 different FAPGG concentrations. Then, the Lineweaver–Burk plot was drawn using these values (Fig. 4). From this graph, V_max and K_M values were calculated as 35.59 (μmol/min). mL⁻¹ and 0.18 mM, respectively (Table 2). In a study by Quassinti et al. the V_max and K_M values of the FAPGG substrate for ACE purified from pig serum were determined to be 0.061 ± 0.0014 nmol/min and 0.793 ± 0.052 mM, respectively [32]. In another work, FAPGG substrate for ACE purified from frog ovaries (Rana esculenta) was used. The V_max and K_M values were calculated to be 0.915 ± 0.04 nmol/min and 0.608 ± 0.07 mM, respectively [49]. For the ram epididymal fluid form of the germinal ACE enzyme derived from the sperm membrane, the K_M and V_max values of the FAPGG substrate were calculated as 0.18 mM and 34 μmoles/(min.mg) [55].

In our work, the optimum pH and optimum temperature range at which the activity of ACE purified from sheep lung is the highest was determined. Optimum pH and optimum temperature for ACE purified from sheep lung were found to be pH 7.4 and 40 °C, respectively (Fig. 5 and Fig. 6). Similarly, in our previous work, the optimum pH value of the ACE enzyme purified from human plasma was found between 7.4 and the optimum temperature between 35–40 °C [56]. In another work, ACE was purified from rabbit lung and the optimum temperature was 37 °C and the optimum pH was 8.0–8.3 [57].

Here, the inhibition effect of captopril, a specific ACE inhibitor, on purified from sheep lung investigated. The inhibitory effect of captopril on ACE activity purified using different concentrations of captopril was measured. The
experiments were repeated three times and the experimental error bars were plotted (Fig. 7). % activity versus inhibitor plotted (Fig. 8). Also, measurements were made on the ACE activity at 3 different captopril and 5 different FAPGG substrate concentrations. After, the Lineweaver–Burk graph was drawn with these values (Fig. 9). The inhibition type, Ki, and IC50 values of captopril on purified ACE from these graphs were determined. IC50 value of captopril was found as 0.51 nM. The inhibition type of captopril was found as non-competitive from the Lineweaver–Burk chart and the Ki value was 0.39 nM (Table 2). In our previous study, the inhibition effect of lisinopril on ACE purified from human plasma was studied and the IC50 value was found to be 0.781 nM [58]. In many studies, it has been observed that thiol-containing inhibitors such as captopril can exhibit non-competitive inhibitory effects by forming strong bonds with zinc near the enzyme’s active site. In a study, two inhibitors containing thiol groups [captopril or SQ 14 225 and 2-(2’-hydroxyphenyl)-3-(3-mercaptopropanoyl)-4-thiazolidine carboxylic acid or SA 446] showed both a mixed competitive and non-competitive inhibitory effect on the rat lung ACE enzyme [59]. At the same time, although inhibitors such as captopril, enalaprilat, and ramiprilat are defined as competitive inhibitors, they have shown a mixed and non-competitive inhibitory effect in some studies [60].

Herein, ACE was purified and characterized for the first time from sheep lung by affinity chromatography

Table 2 Comparison scheme of molecular weight, optimum pH, optimum temperature, Vmax, and KM values of purified angiotensin-converting enzyme (ACE)

| Purification                  | The molecular weight | Optimum pH | Optimum temperature | KM values | Vmax values                   | References |
|------------------------------|----------------------|------------|---------------------|-----------|-------------------------------|------------|
| Sheep lung                   | 60 and 70 kDa        | 7.4        | 40 °C               | 0.18 mM   | 35.59 (µmol/min).mL⁻¹         | This study |
| Human plasma                 | 60 and 70 kDa        | 7.4        | 35–40 °C            | –         | –                            | [56]       |
| Rabbit lung                  | –                    | 8.0–8.3    | 37 °C               | 1.8 mM    | 0.42 µmol/min                 | [57]       |
| Adult houseflies (Musca domestica) | 67 kDa              | –          | –                   | 235 ± 21 µM | 88 ± 4 units/mg protein       | [31]       |
| Swine serum                  | 180 kDa              | –          | –                   | 0.793 ± 0.052 mM | 0.061 ± 0.0014 nmol/min | [32]       |
| Frog ovary (Rana esculenta)  | 150 kDa              | 7–8.5      | 50 °C               | 0.608 ± 0.07 mM | 0.915 ± 0.04 nmol/min | [49]       |
| Sperm membrane-derived germinal ACE | 94 kDa              | –          | –                   | 0.18 mM   | 34 µmoles/(min.mg)            | [55]       |
Molecular weight, purity, and optimum conditions of the purified ACE enzyme were determined. ACE was successfully purified in single-step with NHS-activated Sepharose 4 fast flow column filler without using detergent, ultrafiltration, precipitation with neutral salts, dialysis. The molecular weight and purity of the purified enzyme were confirmed with the SDS-PAGE method. In this method, proteins are separated into subunits where they are denatured with SDS detergent. It was observed that the ACE enzyme purified from sheep lung was separated with SDS-PAGE into two subunits of 60 and 70 kDa. This showed that the ACE enzyme is a dimer enzyme with two sub-monomers.

ACE associated with the vascular endothelium is one of the most extensively studied enzymes both in vivo and in vitro, partly due to its contribution to the continuation of blood pressure in normal subjects and the pathogenesis of systemic hypertension in animal models and humans [10, 11]. The ACE enzyme is an important enzyme that regulates blood pressure and ACE inhibitors are generally utilized in hypertension treatment. Therefore, doing inhibitor research on pure ACE activity gives more accurate results. In future studies, many studies can be done on these inhibitors with the pure ACE enzyme.

Nowadays, the importance of ACE has increased even more. Because the ACE2 enzyme, which is a homolog of the ACE enzyme, is the entry point into the cells of violent acute respiratory syndrome coronavirus 2 (SARS-CoV-2, COVID19). ACE2, which is expressed in various human organs, catalyzes angiotensin II conversion to vasodilator angiotensin-(1–7) [61]. In studies on rodents, it has been observed that ACE inhibitors utilized in hypertension treatment increase the amount of ACE2 and therefore may increase the severity of coronavirus infections. There are concerns that ACE inhibitors increase susceptibility to the coronavirus SARS-CoV-2 virus and the severity of COVID19 disease. However, no direct studies have been found to show that ACE inhibitors increase the risk of COVID19 [62–64].

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Author contributions VT contributed to the study conception and design. All authors contributed to the manuscript. VT, FA, and ZB performed the experiments and analyzed the data. ZB wrote the first draft of the manuscript. VT and ZB contributed to the revisions and the final draft of the manuscript. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.
Ethical approval  The proposed research project detailed above does not need Animal Researches Ethics Committee Approval. Date: 03/10/2019 Decision number: 2019/09.

References

1. Baradaran A, Nasri H, Rafieian-Kopaei M (2014) Oxidative stress and hypertension: possibility of hypertension therapy with antioxidants. J Res Med Sci 19(4):358–367
2. Bakris GL, Toto RD, McCullough PA, Rocha R, Purkayastha D, Davis P (2008) Effects of different ACE inhibitor combinations on albuminuria: results of the GUARD study. Kidney Int 73(11):1303–1309
3. Yang HY, Erdös EG, Levin YA (1970) dipeptidyl carboxypeptidase that converts angiotensin I and inactivates bradykinin. Biochim Biophys Acta 214:374–376
4. Rousseau A, Michaud A, Chauvet MT, Lenfant M, Corvil P (1995) The homoregulatory peptide N-acetyl-Ser-Asp-Lys-Pro Is a natural and specific substrate of the N-terminal active site of human angiotensin-converting enzyme. J Biol Chem 270:3656–3661
5. Deddish PA, Marcie B, Jackman HL, Wang HZ, Skidgel RA, Erdös EG (1998) N-domain-specific substrate and C-domain inhibitors of angiotensin-converting enzyme: angiotensin-1(1–7) and keto-ACE. Hypertension 31:912–917
6. Skidgel RA, Engelbrecht S, Johnson AR, Erdös EG (1984) Hydrolysis of substance P and neurotensin by converting enzyme and neutral endopeptidase. Peptides 5:769–776
7. Natesh R, Schwager SL, Sturrock ED, Acharya KR (2003) Crystal structure of the human angiotensin-converting enzyme-lisinopril complex. Nature 421(6922):551–554
8. Hangauer DG, Monzingo AF, Matthews BW (1984) An interactive computer graphics study of thermolysin-catalyzed peptide cleavage and inhibition by N-carboxymethyl dipeptides. Biochemistry 23(24):5730–5741
9. Sturrock ED, Natesh R, van Rooyen JM, Acharya KR (2004) Structure of angiotensin I-converting enzyme. Cell Mol Life Sci 61(21):2677–2686
10. Cushman DW, Cheung HS (1971) Concentrations of angiotensin-converting enzyme in tissues of the rat. Biochem et Biophys Acta (BBA)—Enzymol 250(1):261–265. https://doi.org/10.1016/0005-2744(71)90142-2
11. Meng QC, Oparil S (1996) Purification and assay methods for angiotensin-converting enzyme. J Chromatogr A 743(1):105–122
12. Campbell DJ (1987) Circulating and tissue angiotensin systems. J Clin Invest 79:1–6
13. Defendini R, Zimmerman EA, Weare JA, Alhenc-Gelas F, Erdös EG (1983) Angiotensin-converting enzyme in epithelial and neuroepithelial cells. Neuroendoocrinology 37:32–40
14. de Andrade MCC, Di Marco GS, de PauloCastroTeixeira V, Mortara RA, Sabatini RA, Pesquero JB, Boim MA, Carmona AK, Schor N, Casarini DE (2006) Expression and localization of N-domain ANG I-converting enzymes in mesangial cells in culture from spontaneously hypertensive rats. Am J Physiol Renal Physiol 290:F364–F375
15. Cushman DW, Cheung HS (1971) Concentrations of angiotensin-converting enzyme in tissues of the rat. Biochem Biophys Acta 250:261–265
16. Takada Y, Hiwada K, Kokubu T (1981) Isolation and characterization of angiotensin converting enzyme from human kidney. J Biochem 90:1309–1319
17. Chappell MC, Diz DJ, Gallagher PE (2001) The renin-angiotensin system and the exocrine pancreas. JOP 2:33–39
18. Lattion AL, Soubrier F, Allegrini J, Hubert C, Corvol P, Alhenc-Gelas F (1989) The testicular transcript of the angiotensin I-converting enzyme encodes for the ancestral, non-duplicated form of the enzyme. FEBS Lett 252:99–104
19. Deddish PA, Wang J, Michel B, Morris PW, Davidson NO, Skidgel RA, Erdös EG (1994) Naturally occurring active N-domain of human angiotensin I-converting enzyme. Proc Natl Acad Sci USA 91:7807–7811
20. Kokubu T, Kato I, Nishimura K, Hiwada K, Ueda E (1978) Angiotensin I-converting enzyme in human urine. Clin Chim Acta 89:375–379
21. Casarini DE, Plavnik FL, Zanella MT, Marson O, Krieger JE, Hirata JY, Stella RC (2001) Angiotensin converting enzymes from human urine of mild hypertensive untreated patents resemble the N-terminal fragment of human angiotensin I-converting enzyme. Int J Biochem Cell Biol 33:75–85
22. Erdös EG (1990) Angiotensin I converting enzyme and the changes in our concepts through the years Lewis K. Dahl memorial lecture. Hypertension 16:363–370
23. Casarini DE, Carmona AK, Plavnik FL, Zanella MT, Juliano L, Ribeiro AB (1995) Calcium channel blockers as inhibitors of angiotensin I-converting enzyme. Hypertension 26:1145–1148
24. Marques GD, Quinto BM, Plavnik FL, Krieger JE, Marson O, Casarini DE (2003) N-domain angiotensin I-converting enzyme with 80 kDa as a possible genetic marker of hypertension. Hypertension 42:693–701
25. Skidgel RA, Erdös E (1993) Biochemistry of angiotensin I-converting enzyme. In: Robertson JIS, Nichols MG (eds) The renin-angiotensin system. Raven Press Ltd, New York
26. Brown NJ, Vaughan DE (1998) Angiotensin-converting enzyme inhibitors. Circulation 97:1411–1420
27. Hanif K, Bid HK, Konwar R (2010) Reinventing the ACE inhibitors: some old and new implications of ACE inhibition. Hypertens Res 33:11–21
28. Urh M, Simpson D, Zhao K (2009) Affinity chromatography: general methods. Methods Enzymol 463:417–438
29. Bull HB, Thornberry NA, Cordes EH (1985) Purification of angiotensin converting enzyme from rabbit lung and human plasma by affinity chromatography. J Biol Chem 260:2963–2972
30. Hooper NM, Keen J, Pappin DJC, Turner AJ (1987) Pig kidney angiotensin converting enzyme. Purification and characterization of amphiaphatic and hydrophilic forms of the enzyme establishes C-terminal anchorage to the plasma membrane. Biochem J 247:85–93
31. Lamango NS, Sajid M, Isaac RE (1996) The endopeptidase activity and the activation by Cl– of angiotensin-converting enzyme is evolutionarily conserved: purification and properties of an angiotensin-converting enzyme from the housefly, Musca domestica. Biochem J 314:639–646
32. Quassinti L, Miano A, Bramucci M, Maccari E, Amici D (1998) Purification of swine serum angiotensin converting enzyme with high recovery of activity using lisinopril coupled to epoxy activated sepharose 6B. Biochem Mol Biol Life Sci 44(5):887–895
33. Andújar-Sánchez M, Cámara-Artigas A, Jara-Pérez V (2003) Purification of angiotensin I converting enzyme from pig lung using concanavalin-A sepharose chromatography. J Chromatogr B 783:247–252
34. Pantoliano MW, Holmquist B, Riordan JF (1984) Affinity chromatographic purification of angiotensin converting enzyme. Biochemistry 23(5):1037–1042
35. Sabeur K, Vo AT, Ball BA (2001) Characterization of angiotensin-converting enzyme in canine testis. Reproduction 121(1):139–146
36. Holmquist B, Bünning P, Riordan JF (1979) A continuous spectrophotometric assay for angiotensin converting enzyme. Anal Biochem 95(2):540–548
37. Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
38. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685
39. Lineuweaver B, Burk D (1934) The determination of enzyme dissociation constants. J Am Chem Soc 56:658–660
40. Aragão DS, de Andrade MCC, Ebihara F, Watanabe IK, Magalhães DC, Juliano MA, Hirata IY, Casarini DE (2015) Serine proteases as candidates for proteolytic processing of angiotensin-I converting enzyme. Int J Biol Macromol 72:673–679
41. Basi Z, Turkoglu N, Karahan F (2019) In vitro effect of ethyl acetate, butanol and water extracts of Juniperus excelsa Batter. on angiotensin converting enzyme purified from human plasma. Chem Pap 73:2525–2533
42. Bas Z, Turkoglu V, Goz Y (2021) Investigation of inhibition effect of butanol and water extracts of Matricaria chamomilla L. on angiotensin-converting enzyme purified from human plasma. Biotechnol Appl Biochem. https://doi.org/10.1002/bab.2106
43. Basi Z, Turkoglu V (2019) In vitro effect of oxidized and reduced glutathione peptides on angiotensin converting enzyme purified from human plasma. J Chromatogr B Analyt Technol Biomed Sci 104:190–195
44. Mendelsohn FA, Csicsmann J, Hutchinson JS (1981) Complex competitive and non-competitive inhibition of rat lung angiotensin-converting enzyme by inhibitors containing thiol groups: capttopril and SA 446. Clin Sci 61:277s–280s
45. Dietz LH, Bénéteau-Burnat B (1999) Mixed-type inhibition of mixed-type angiotensin I-converting enzyme by captopril, enalaprilat and ramiprilat. J Enzym Inhib 14(6):447–456
46. Santos RAS, Sampaio W, Alzamora A.C., Motta-Santos, D., Alenina N., Bader, M., & Campagnole-Santos, M. J. (2018) The ACE2/angiotensin-(1–7)/MAS axis of the renin-angiotensin system: focus on angiotensin-(1–7). Physiol Rev 98:505–553
47. Nicholls J, Peiris M (2005) Good ACE, bad ACE do battle in lung injury SARS. Nat Med 11(8):821–822
48. Diaz JH (2020) Hypothesis: angiotensin-converting enzyme inhibitors and angiotensin receptor blockers may increase the risk of some active ingredients isolated from Nigella sativa L. extract on the enzyme activity. Biomed Chromatogr 32(5):4175
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