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ORIGINAL ARTICLE

Alu insertion/deletion of *ACE* gene polymorphism might not affect significantly the serum bradykinin level in hypertensive patients taking ACE inhibitors

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Abstract *Background:* Angiotensin I-converting enzyme (ACE) has two homologous catalytic domains, the N- and C-domains. Our previous study suggested that *Alu* insertion (I allele) in the intron 16 of ACE resulted in premature codon termination. The I allele has only one active site in the N-domain while the *Alu* deletion (D allele) still has two active sites of ACE. Therefore the effect of I/D polymorphism of ACE on the enzyme's ability to catalyse bradykinin is still not widely known.

Aims: This study aimed to examine the serum bradykinin level in hypertensive patients with I/D polymorphism of ACE, who were treated with ACE inhibitor.

Subjects and methods: The serum bradykinin and I/D polymorphism have been detected in 64 hypertensive patients taking ACE inhibitor (lisinopril or captopril) for at least eight weeks with good medication adherence. The binding affinity of ACE with its receptor was calculated by molecular docking.

Results: The findings show that genotype II is more frequent in the population the researchers observed (53.12%) compared to ID (23.44%) and DD (23.44%) variances. On the other hand, the bradykinin level is not affected by genotype of the ACE genes on the population. Bradykinin increases in

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patients with genotype II who are given captopril, but decreases in patients treated with lisinopril. Nevertheless, there is no statistically significant difference.

Conclusion: This study suggests that the polymorphism might not significantly affect the serum bradykinin level in hypertensive patients taking ACE inhibitors.

1. Introduction

Angiotensin I-converting enzyme (ACE) is a zinc metallopeptidase that plays an important role in blood pressure regulation [1–3]. The ACE has two homologous catalytic domains, the N- and C-domains, which are able to cleave angiotensin I and bradykinin [4,5,2]. The C-domain of ACE is more efficient in cleaving angiotensin I into vasopressor angiotensin II [4]. However, reports regarding the bradykinin binding site to the ACE are still limited. This information is necessary to measure the strength of binding competition between bradykinin and the angiotensin I to the ACE, although the ACE inhibitors generally have a higher affinity for the bradykinin than the angiotensin I binding sites [5]. This knowledge is essential for developing more specific ACE inhibitors on angiotensin I binding site as a hypertension drug that has no side effects on cough.

Our previous study suggested that *Alu* insertion (I) in the intron 16 of ACE resulted in premature codon termination, so the protein has only one active site in the N-domain while the *Alu* deletion allele (D) still has two active sites. The meta-analysis indicated that the D allele is related to higher levels of angiotensinogen that is associated with metabolic syndrome [6], while the I allele of ACE correlated with the development of Alzheimer's disease [7]. Moreover, the I/D polymorphism in the ACE gene has been linked to several kinds of diseases, such as coronary artery disease [8], infections in post-operative cardiac valve surgery patients [9] and arterial hypertension [10].

Therefore information of the effect I/D polymorphism on ACE activity in catalysing bradykinin is still limited. Further, we have examined the serum bradykinin level in hypertensive patients with I/D polymorphism of ACE, who were treated with ACE inhibitor. This study suggests that the polymorphism does not significantly influence the serum bradykinin level of hypertensive patients who are taking ACE inhibitors.

2. Subjects and methods

2.1. Subjects and detection of serum bradykinin level

The study recruited 64 hypertensive patients, over 18 years old, who had been taking an ACE inhibitor (lisinopril or captopril) for at least eight weeks with good medication adherence, measured by the Morisky Medication Adherence Scale (MMAS). Patients with secondary hypertension, cardiovascular diseases, liver dysfunction, and were currently receiving treatment with angiotensin receptor blockers (ARBs) were excluded from this study. A blood sample was taken to detect ACE polymorphism and measure bradykinin level. The level of bradykinin serum was measured according to the manufacturer's instructions (Cusabio Biotech; Cat. No. CSB-E09155h), the levels of bradykinin serum were examined using Analysis of Variance and quantitative variables were expressed as mean ± standard deviation values. A value of $p \leqslant 0.05$ was considered as statistically significant. This study has followed the Code of Ethics of the world Medical Association (Declaration of Helsinki) for experiments in humans and approved by Brawijaya University/RSSA hospital ethics.

2.2. Polymorphism detection

Genomic DNA samples were isolated by DNA extraction kit (Geneaid™). Then the genomic DNA was used as a template to amplify a DNA fragment on intron 16 of the ACE gene. The amplification was done by polymerase chain reaction (PCR), using a forward primer 5′-GCC CTG CAG GTG TCT GCA GCA TGT-3′ and reverse 5′-GGA TGG CTC TCC CCG CCT TGT CTC-3′. Thirty-four cycles of PCR were performed with the following parameters: denaturation at 96 °C for 45 s, annealing at 60.3 °C for 45 s, and extension at 72 °C for 45 s. The D allele is characterised by a 312-bp fragment, whereas 599-bp fragment indicates I allele. Each sample found to have the DD genotype was subjected to the second PCR to amplify a region inside intron 16 with insertion-specific primers 5′-TGG GAC CAC AGC GCC CGC CAC TAC-3′ and 5′-TCG CCA GCC CTC CCA TGC CCA TAA-3′ to avoid ID-DD mistyping [9].

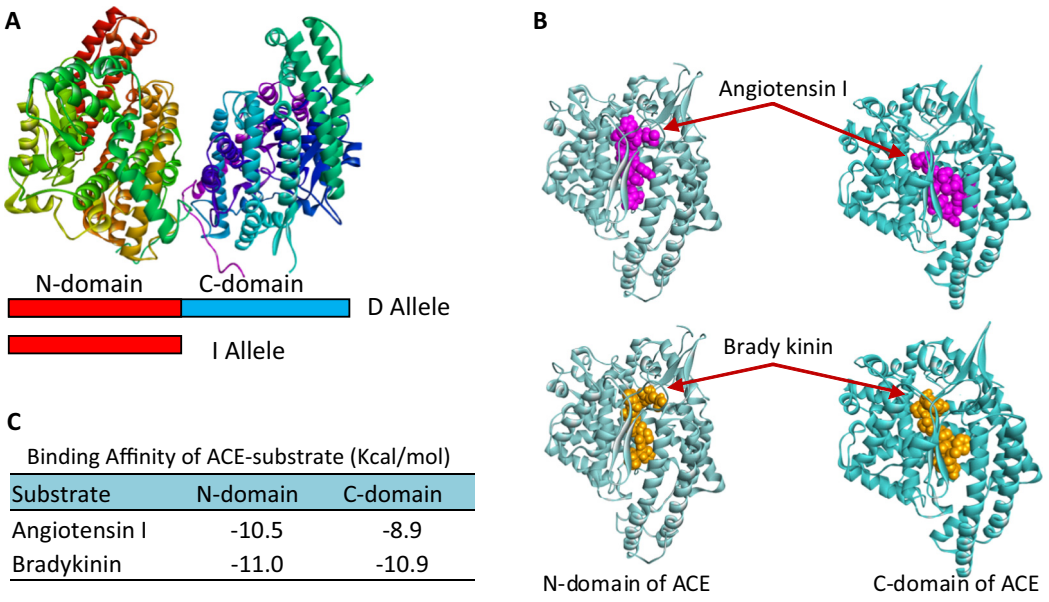
2.3. Molecular docking

The ACE molecule was retrieved from Protein Data Bank in Europe (PDBe) with access code 5AMB [11]. The simplified molecular input line entry system (SMILES) structure of bradykinin and angiotensin I was taken from PubChem [12], then they were converted into PDB format using Discovery Studio Software [13]. Lisinopril and the N-domain of ACE were extracted from PDB (2C6N) [14], while captopril and the C-domain of ACE were retrieved from PDB (1UZF) [15]. Molecular docking between ACE and its ligands (lisinopril, captopril, bradykinin and angiotensin I) were performed by using AutoDock Vina, PyRx software [16]. The predicted binding affinity (kcal/mol) was calculated by AutoDock Vina. All molecules were visualised by Discovery Studio [13].

3. Results

3.1. Interaction of ACE with bradykinin and angiotensin I

ACE has two domains – the N- and C-terminals [3–5]. Both domains work independently and alternately when they catalyse their substrates [17]. ACE with *Alu* insertion (I) in the intron 16 was predicted to lose the C-domain, while the Deletion allele(D) still has two active sites [18]. Furthermore, dock-

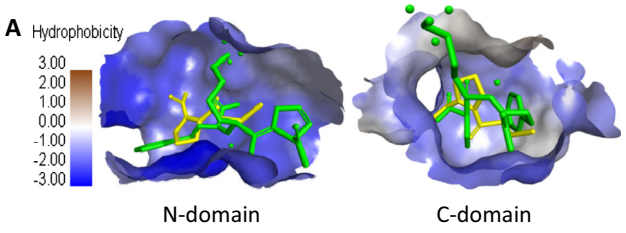


C

Binding Affinity of ACE-substrate (Kcal/mol)

Substrate	N-domain	C-domain
Angiotensin I	-10.5	-8.9
Bradykinin	-11.0	-10.9

Figure 1 The interaction between ACE and its substrates. The full-length structure of ACE, which has two domains (N- and C-domains) (5AMB), *Alu* element insertion (I allele) might induce premature termination that results in the loss of the C-domain (A). Bradykinin and angiotensin I have the same binding site in both the N- and C-domains (B). Bradykinin has an equal binding affinity in both the N- and C-domains, but angiotensin I looks more strong binding in the N-domain rather than in the C-domain (C).



B The binding affinity between ACE and its inhibitor

Binding Affinity (Kcal/mol)	N-Domain	C-Domain
Captorpil	-5.9	-6.1
Lisinopril	-7.1	-8.3

Figure 2 Binding Position between ACE and its inhibitors. Captopril (yellow) and lisinopril (Green) bind with ACE (N- and C-domains) (A); It is predicted that captopril binds as strongly in both domains while lisinopril tends to have a stronger binding in C-domain (B).

ing analysis was conducted to find the binding affinity between N- and C-domains with their substrates, bradykinin and angiotensin I. The result of the analysis shows that bradykinin has a similar binding affinity towards the N- and C-domains of ACE (Fig. 1). This is in line with the previous study, which states that the bradykinin level is equivalent in the N-domain or C-domain of mice [3]. Conversely, angiotensin I has a smaller binding affinity towards the N-terminal compared to C-terminal.

Furthermore, we analysed the binding affinity of the N- and C-domains of ACE with captopril or lisinopril. The study examined the inhibitors' preference to block ACE activities. The binding affinity is essential to determine the potency of the inhibitor in controlling the variation of ACE (ID) activity in order to catalyse bradykinin. Analysis using molecular docking shows that captopril has a similar binding affinity in both domains, which indicates captopril is efficient in binding to both domains. However, lisinopril has a smaller binding affinity in the C-domain than in the N-domain. This means lisinopril binds more efficiently in the C-domain. Both molecules are attached to the active side of the two ACE domains (Fig. 2).

3.2. Correlation between genotype of ACE and serum bradykinin level

The baseline characteristics of hypertensive patients from two groups – patients who were treated with lisinopril and patients who were treated with captopril – showed no significant differences. The measured characteristics were age, sex, Systolic Blood Pressure (SBP; mmHg), Diastolic Blood Pressure (DBP; mmHg), blood glucose and cholesterol. Among the 64 hypertension patients, 34 were found to have genotype II, and 30 patients had DD/DI genotype.

We have measured the level of serum bradykinin of 64 hypertensive patients who were treated with an ACE inhibitor, either captopril or lisinopril. The findings show that ACE II variance is found more frequently in the population (53.12%) compared to DI (23.44%) and DD (23.44%) variance (Table 1).

On the other hand, the level of bradykinin is barely influenced by the genotype of the ACE genes. Even though the level of bradykinin increases in genotype II of the patients treated using captopril, its level decreases in patients treated with lisinopril. However, there is no statistically significant difference.

4. Discussion

Angiotensin-converting enzyme (ACE) is an enzyme that plays a crucial role in controlling blood pressure by the cleavage of

Table 1 The serum bradykinin level (pg/ml) in patients receiving lisinopril and captopril.

ACE inhibitor	ACE genotype			*P*-value
	DD	DI	II	
Captopril	4.12 ± 3.03 (n = 7)	4.30 ± 3.00 (n = 8)	6.47 ± 5.65 (n = 17)	0.40
Lisinopril	5.59 ± 4.02 (n = 8)	5.87 ± 2.01 (n = 7)	4.79 ± 4.53 (n = 17)	0.88

*p value $\leqslant$ 0.05 = significantly different between groups; n = number of subject.

angiotensin I into angiotensin II, which triggers vasoconstriction. In addition, ACE also inactivates bradykinin, a substance of the function of which is to increase vasodilatation in the body. Thus, ACE works simultaneously to catalyse angiotensin I and bradykinin [2,3]. ACE has two independent catalytic domains [3–5]. The domains are capable of cleaving angiotensin I and bradykinin but differ in binding affinity [14]. The results of the docking analysis show that the binding affinity of angiotensin I to the N-domain is stronger to the C-domain, while the binding affinity of bradykinin is almost equal to both the N- and C-domain. Moreover, the binding affinity of ACE to bradykinin is stronger than that of angiotensin I. It can be concluded that ACE more preferably binds to bradykinin than angiotensin I, therefore, the inhibition of the bradykinin binding site on ACE is a promising target for developing antihypertension drugs. These data correspond with the previous study that demonstrated that ACE inhibitor has a higher binding affinity towards bradykinin than it has towards angiotensin I binding site [5]. Hence, ACE is the primary target for drugs used to control hypertension [19].

ACE gene variance due to insertion or deletion of *Alu* elements is predicted to have an influence on bradykinin catalysis. Further analysis shows that bradykinin binds equally strongly to the N- and C-domains of ACE. The data indicate that both ACE domains have a similar quality in bradykinin catalysis. This is in line with the previous study that found that deletion on either the N- or C-domain of ACE does not affect the level of bradykinin in the mice [3]. Thus, the ability of ACE to inactivate bradykinin is not affected by insertion or deletion. This is supported by the study that found that the bradykinin level in the serum of genotype II and DD does not show a significant difference. This is consistent with other studies in Japanese hypertensive patients receiving an ACE inhibitor [17,18].

Although ACE genetic variance does not influence the level of bradykinin significantly, the bradykinin level tends to be affected by ACE inhibitor therapy used by patients. ACE inhibitor reduces the ACE activity that may lead to the accumulation of serum bradykinin. The level of bradykinin tends to increase in patients treated with captopril and, conversely, to decrease in patients treated using lisinopril, even though statistically there was no significant difference. This condition might be caused by the large standard deviation as a result of the low sample number. Therefore, adequate samples are needed to analyse the statistical significance of *ACE* gene I/D variant with the level of bradykinin serum.

The phenomenon indicated that ACE inhibitor influenced serum bradykinin levels. This can be seen from the binding affinity between captopril and lisinopril in each ACE domain. Lisinopril has a higher binding affinity for the C-domain of ACE, which indicates that lisinopril inhibits the C-domain of ACE more efficiently than the N-domain. These data correspond with *in vitro* data that show that the inhibitory constant (Ki) of lisinopril is lower in the C-domain than in the N-domain [20]. This phenomenon is supported by our serum bradykinin level data. The serum bradykinin level in hypertensive patients with ID/DD genotype receiving lisinopril tended to be higher than in those with II genotype, although the difference is not statistically significant. Captopril was bound to N- and C-domains with similar affinity. The data are analogous to a previous study that found that captopril has a similar binding affinity in the two domains [21]. This result means that captopril could bind into the two ACE domains, but its inhibitory potency is higher in the N-domain than in the C-domain. Interestingly, the level of bradykinin serum in patients receiving captopril was greater in the II genotype than in the DD genotype. Because I allele might have only the N-domain, captopril could bind effectively, so the I variant showed a slightly higher serum bradykinin serum level than the D allele.

5. Conclusion

The binding affinity of bradykinin was equal in both the C-and N-domains of ACE. There was no statistical difference in the level of bradykinin in the II and DD genotype of a hypertensive patient treated with ACE inhibitor, captopril or lisinopril.

Ethics

The study was approved by the local committee on medical faculty of Brawijaya University and RSSA Hospital. Written informed consent was obtained from all study participants.

Conflict of interest

None.

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