생 약 학 회 지
Kor. J. Pharmacog.
12(1) : 51 ~ 54 (1981)

Screening of Plant Materials for the Inhibitory Activities Against Angiotensin Converting Enzyme

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植物生藥의 안지오텐신變換酵素 抑制作用 檢索

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Twenty-seven medicinal plants were selected on the basis of folkloric reputation for the treatment of hypertension or related deseases. Two solvent fractions were prepared from methanol extract of each plant and tested for their effects on angiotensin converting enzyme (ACE) activities. Six solvent fractions showed more than 50% inhibition and four showed 40~50% inhibition at the conditions tested.

Angiotensin converting enzyme (ACE, peptidyldipeptidase hydrolase, EC 3.4.15.1) is an exopeptidase which cleaves dipeptides from the carboxy-terminal end of various peptide substrates. ACE plays an important role in blood pressure regulation by catalyzing two important reactions (Fig. 1): a) conversion of the inactive decapeptide angiotensin I to the potent vasopressor octapeptide angiotensin II[1] and b) inactivation of the vasodepressor nonapeptide bradykinin to inactive fragments.[2]

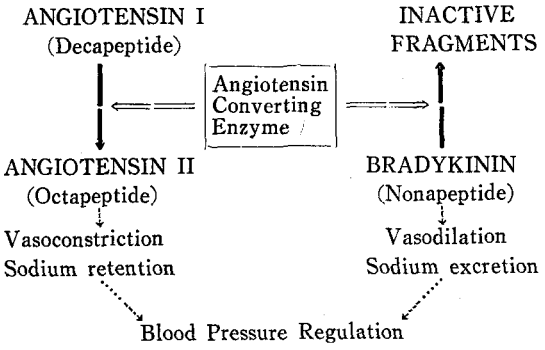

Fig. 1. Some reactions catalyzed by angiotensin converting enzyme.

In the last few years, it has been demonstrated that inhibition of this enzyme (ACE) might lower blood pressure in animal models and in human with various forms of hypertension[3,4,5] and various compounds have been prepared and tested as inhibitors of ACE.[6~10] Most of them are either peptide or peptide analogs.

The present work is aimed to investigate potential non-peptide inhibitors of ACE from plant sources. Plants were selected with folkloric reputation for the treatment of hypertension or related deseases. The effects of plant materials on ACE activities were measured. Several of them showed high potential of containing potent ACE inhibitors.

Experimental Procedure

Plant samples: Plant samples were purchased from the local herb drug market and

identified taxonomically.

Extraction and solvent fractionation of plant samples: Plant samples were extracted and fractionated as described on Fig. 2. 300g of each plant sample was extracted twice with methanol for 6 hrs. Then it was filtered off and the filtrate was concentrated under reduced pressure. The MeOH extract was partitioned between $CHCl_3$ and water. $CHCl_3$ layer on evaporation of the solvent was again partitioned with hexane and 90% MeOH. Water layer (Fr. I) and 90% MeOH layer (Fr. II) after evaporation of the solvents were tested for their effects against ACE activities.

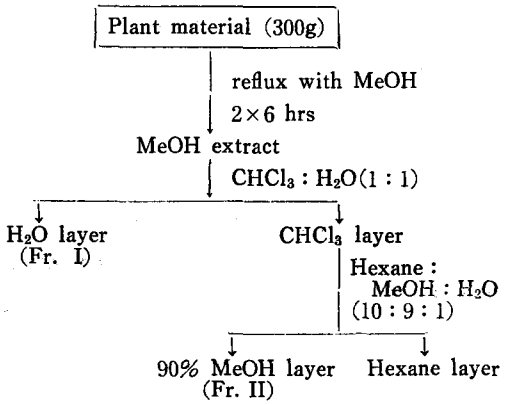

Fig. 2. Extraction and solvent fractionation of plant materials.

Enzymatic studies: Hippuryl-L-histidyl-L-leucine (Hip-His-Leu) and acetone powder of rabbit lung were purchased from Sigma Chemical Company Inc., U.S.A. Crude enzyme preparation from rabbit lung acetone powder and spectrophotometric assay of the hydrolysis of Hip-His-Leu by ACE were performed as described by Cushman and Chung[11] with minor modifications.

Rabbit lung acetone powder(1g) homogenized with 20ml of 50mM potassium phosphate buffer, pH 8.3, was centrifuged for 40min. at 40,000g and the clear supernatant was used as stock enzyme solution. The stock enzyme solution, diluted with equal volume of 150mM potassium phosphate buffer with 600mM NaCl, pH 8.3, was used for enzymatic evaluation. Each enzyme assay consists of 0.25ml containing the following components at the final concentrations: 100mM potassium phosphate buffer, 300mM NaCl, 4.6 mM sodium-Hip-His-Leu and approximately 5mμ of the enzyme. The enzyme, in a volume of 0.15ml or less, was added last to initiate the reaction and the tubes were incubated for 30 min. in a Dubnoff metabolic shaking incubator. The enzymatic reactions were terminated by addition of 0.25ml of 1N HCl. The reaction mixture was shaked with ethyl acetate (3ml) and centrifuged for 10min. at 8,500g. A 2.0ml aliquot of each ethyl acetate layer was taken and the solvent was evaporated off. The remaining hippuric acid was dissolved in 1ml water and the amount formed was determined from its absorbance at 228mμ.

The effects of plant preparations (Fr. I or Fr. II of Fig. 2) were measured at the final concentrations of 0.2mg/ml. Each sample was run four times and the average was calculated as % inhibition value.

Results and Discussions

Plants were selected from those which have been used for hypertension of related deseases in the form of oriental medicines or folkloric remedies in Korea. Total of 54 plant preparations (Fr. I and Fr. II of 27 plant samples) were tested for their effects on ACE activities wheather they exert antihypertensive effects if at all through their inhibitory effects on ACE. Hexane fr. (Fig. 2) was not tested in the present experiment because of the solubility problems. The results are summarized in Table 1. Six preparations showed more than 50% inhibition at the conditions tested and four showed

40～50% inhibition. 14 preparations showed no effects. The activities of the plant preparations might due to specific inhibitory effects exerted by one on more of the constituents or due to non-specific enzymic inactivation. This should be further clarified.[12,13] However, in the present experiment, it is shown that plants could serve as a potential source for separation of compounds with inhibitory effects of ACE.

Acknowledgements: The authors are grateful to Dr. Hyung Joon Chi for helping us identifying the plant materials.

Table 1. Effects of plant preparations on ACE activities

Plant name	Family name	Parts of plants used	% Inhibition	
			Fr. I	Fr. II
Acanthopanax spp.	Araliaceae	ba	42	31
Achyranthes japonica	Amarantaceae	ra	—	25
Aconitum pseudolaeve	Ranunculaceae	ra	—	36
Alisma orientale	Alismataceae	tu	—	32
Angelica gigas	Umbelliferae	ra	46	—
Angelica koreana	Umbelliferae	ra	—	—
Aralia continentalis	Araliaceae	ra	—	23
Astragalus membranaceus	Leguminosae	ra	—	16
Atractylodes japonica	Compositae	rh	39	16
Carthamus tinctorius	Compositae	fl	—	36
Chrysanthemum indicum	Compositae	fl	26	—
Conioselium spp.	Umbelliferae	rh	12	70
Crataegus pinnatifida	Rosaceae	fr	50	26
Eucommiae ulmoides	Eucommiaceae	sb	37	51
Fritillaria spp.	Liliaceae	tu	20	48
Inula helenium	Compositae	ra	—	38
Ledebouriella seseloides	Umbelliferae	ra	22	16
Leonurus sibiricus	Labiatae	ha	30	18
Lycium chinense	Solanaceae	fr	—	19
Machilus thunbergii	Lauraceae	sb	17	36
Nepeta japonica	Labiatae	ha	74	20
Paeonia obovata	Ranunculaceae	ra	—	46
Polygonatum japonicum	Liliaceae	rh	54	31
Prunus persica	Rosaceae	sm	35	—
Rehmannia glutinosa	Scrophulariaceae	ra	16	15
Saussurea lappa	Compositae	ra	70	29
Scutellaria baicalensis	Labiatae	ra	22	10

ba: bark, fl: flower, fr: fruit, ha:herba, ra: radix, rh: rhizome, sb: stem bark, sm: semen, tu: tuber.

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