ASTERRIC ACID, A NEW ENDOTHELIN BINDING INHIBITOR

HIROSHI OHASHI, HIROKAZU AKIYAMA, KOJI NISHIKORI and JUN-ICHIRO MOCHIZUKI

Pharmaceutical Research Laboratory, Kirin Brewery Co., Ltd., Takasaki, Gunma 370-12, Japan

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Endothelin (ET) is a potent vasoconstrictor/pressor peptide consisting of 21 amino acid residues isolated from the cultures of porcine aortic endothelial cells[1]. Analysis of a human genomic library reveals three forms of ET (ET-1, ET-2 and ET-3) which have more than 70% homology with one another[2]. In various organs, high affinity and specific receptors for ET have been found[3]. They have been distinguished as receptors ET_A and ET_B[4,5]. ET-1 exhibits potent and long-lasting vasoconstrictive activity *in vivo* and *in vitro*[1], as a result of binding to the ET_A receptor.

In the course of our screening program for ET-1 binding inhibitors from microorganisms, we isolated asterric acid (Fig. 1, **1**) as an active substance from the culture filtrate of a fungus, *Aspergillus* sp. Asterric acid had been reported as a metabolite of a fungus[6,7]. We have shown that it has the property of inhibiting ET-1 binding to the ET_A receptor of A10 cells[8].

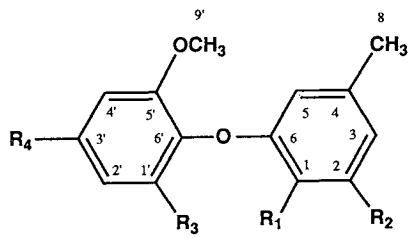
The fungus was cultivated on a rotary shaker at 25°C for 7 days in 500-ml Erlenmeyer flasks each containing 100 ml of K15 medium which consists of 2% malt extract, 2% glucose, 0.1% peptone and 0.1% agar.

The filtrate of the fermentation broth (1 liter) was extracted twice with 500 ml of ethyl acetate at pH 2. The organic layer was concentrated *in vacuo* and subjected to silica gel column chromatography (Wako gel C-200) using $CHCl_3$-MeOH (50:1, 10:1). After evaporation, the active portion was applied to a centrifugal partition chromatograph (ethyl acetate-0.1 M tris-HCl buffer (pH 8.0)) following gel filtration (Sephadex LH20, MeOH) to give 21 mg of pure substance (**1**).

The ^1H NMR spectrum of **1** indicated the presence of four aromatic protons (δ_H in $(CD_3)_2CO$; 5.92 (1H, s), 6.49 (1H, s), 6.93 (1H, d, J=2.9 Hz), 7.06 (1H, d, J=2.9 Hz)), six $-OCH_3$ protons (3.74 (3H, s), 3.82 (3H, s)), three Ar-CH_3 protons (2.17 (3H, s)). The UV_{max} of **1** were 212.8, 248.0 and 313.2 nm in MeOH. FD-MS spectrum of **1** had a parent ion peak at m/z 348 (M^+). These data were consistent with literature values[6,7] of asterric acid. The ^{13}C NMR spectral data and the results of NOE and HMBC experiments supported the identity of **1** and asterric acid. Our assignments are summarized in Table 1.

Table 2 shows the ET-1 binding-inhibition activity of asterric acid. Asterric acid inhibits ET-1 binding to the ET_A receptor of A10 cells (IC_{50} 1.0×10^{-5} M). Since A10 cells also have receptors of atrial natriuretic peptide (ANP) and angiotensin II (A II), the inhibitory effects of asterric acid on their binding

Fig. 1. The structures of asterric acid and its derivatives.



	R_1	R_2	R_3	R_4
1	COOH	OH	$COOCH_3$	OH
2	$COOCH_3$	OCH_3	$COOCH_3$	OCH_3
3	COOH	OH	COOH	OH
4	$CONHNH_2$	OH	$COOCH_3$	OH

Table 1. 125 MHz ^{13}C NMR and 500 MHz ^1H NMR spectral data of **1** (in CD_3OD).

Position	δ_C (ppm)	δ_H (ppm)
1	104.8	
2	164.3	
3	111.8	6.44
4	145.4	
5	106.3	5.78
6	161.1	
7	167.6	
8	21.9	2.14
1'	127.0	
2'	106.2	6.82
3'	136.7	
4'	109.3	6.94
5'	155.3	
6'	156.7	
7'	167.6	
8'	52.7	3.78
9'	56.8	3.72

Table 2. Effect of asterric acid on ET-1 binding to A10 cell.

Asterric acid (μM)	ET specific binding (%)
0.1	100
0.3	99
1.0	92
3.0	77
10	50
30	30
100	11
300	6

A10 cells at 1×10^4 cells/well were incubated with ^{125}I-ET for 6 hours at 4°C. Various concentrations of asterric acid were coincubated during this periods. After incubation, cells were washed and cell-bound radioactivity was counted.

were examined. It showed no inhibitory activity against ANP and A II binding at 10^{-3} M. These results indicate that asterric acid is a specific inhibitor of ET-1 binding.

We prepared some derivatives of asterric acid (Fig. 1). The methylated compound (**2**) was obtained by using NaH-CH_3I. KOH treatment gave demethyl asterric acid (**3**). The compound (**4**) was formed from (**1**) by treatment with chloroethyl formate, followed by hydrazine hydrate. These derivatives showed no ET-1 binding inhibitory activity at 10 μM. In particular, the activity of the demethyl form was less than 1% of that of asterric acid.

KOJIRI *et al.*[9,10] and MIYATA *et al.*[11,12] have identified cyclic pentapeptides from an Actinomycete as ET binding inhibitors. As far as we know, asterric acid and WS009[13] are the first non-peptide ET binding inhibitors to be identified. Asterric acid will be a useful tool to investigate the functions of ET.

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