British Journal of Pharmaceutical Research
13(6): 1-7, 2016, Article no.BJPR.29824
ISSN: 2231-2919, NLM ID: 101631759

SCIENCEDOMAIN *international*
www.sciencedomain.org

Endothelin-1 (1-31) Causes the Migration of Vascular Smooth Muscle Cells through Endothelin ET_A Receptor

Yoji Kyotani[1*], Jing Zhao[1], Kentaro Ozawa[1], Takahiko Nakagawa[2], Francesco A. Bolstad[3] and Masanori Yoshizumi[1]

[1]Department of Pharmacology, Nara Medical University School of Medicine, Kashihara, Nara, Japan.
[2]Industry-Academia-Government Association Promotion Center, Nara Medical University School of Medicine, Kashihara, Nara, Japan.
[3]Department of Clinical English, Nara Medical University School of Medicine, Kashihara, Nara, Japan.

Authors' contributions

This work was carried out in collaboration between all authors. Author YK designed the study, conducted the acquisition, analysis and interpretation of data and drafted most of the manuscript. Author JZ took part in acquisition of data. Author KO revised the manuscript. Authors MY, TN and FAB developed research questions and revised the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2016/29824
Editor(s):
(1) Elena G. Zavyalova, Chemistry Department, Moscow State University, Russia.
Reviewers:
(1) Francesco Saverio Sorrentino, Unit of Ophthalmology, Ospedale Maggiore, Bologna, Italy.
(2) Gordana Kocic, University of Nis, Serbia.
(3) Volodymyr Chernyshenko, Palladin Institute of Biochemistry NAS of Ukraine, Ukraine.
(4) Yahya Elshimali, Charles Drew University of Medicine and Science, USA.
Complete Peer review History: http://www.sciencedomain.org/review-history/16821

Short Research Article

Received 30th September 2016
Accepted 29th October 2016
Published 7th November 2016

ABSTRACT

Endothelin-1 (ET-1) is predominantly expressed in endothelial cells to modulate physiological actions, such as vasoconstriction and cell proliferation. It acts as an autocrine and paracrine factor, and has been reported to be found in increased levels in the blood of patients with hyperlipidemia and atherosclerosis. The excessive proliferation and migration of vascular smooth muscle cells (VSMCs) characterize the progression of atherosclerosis. Thus, ET-1 is currently believed to be an important factor for atherosclerosis. Endothelin-1 (1-31) is a relatively recently discovered form of

**Corresponding author: E-mail: cd147@naramed-u.ac.jp;*

ET and is generated from big ET-1 by chymase, which is predominantly expressed in mast cells. Recently, the elevated concentration of circulating ET-1 (1-31) in patients with acute myocardial infarction has been reported. In this study, we investigated whether ET-1 (1-31) could induce VSMC migration and compared its effect with that of ET-1. ET-1 (1-31) significantly stimulated rat aortic vascular smooth muscle cell (RASMC) migration in a concentration dependent manner. ET-1 (1-31) at 100 nM caused a 1.38-fold increase in RASMC migration whereas ET-1 at the same concentration resulted in a 1.60-fold increase. The ET-1 (1-31)-stimulated RASMC migration was significantly inhibited by BQ123, a specific ET_A receptor antagonist, but not by BQ788, a specific ET_B receptor antagonist. These data suggest that ET-1 (1-31) stimulates the VSMC migration through ET_A receptors but not ET_B receptors. The findings presented in this paper bring us one step closer to understanding the mechanisms involved in atherosclerosis.

Keywords: Endothelin-1; endothelin-1 (1-31); ET_A receptor; ET_B receptor; rat aortic smooth muscle cell; migration.

1. INTRODUCTION

The Endothelin (ET) family consists of 21 amino acids and is classified into three members, i.e. ET-1, -2 and -3. ET-1 is then initially synthesized as 212-residue precursor peptide, preproET-1 [1], which is processed to a 38-amino acid precursor, big ETs. Subsequently, big ET-1 is catalyzed by ET converting enzyme-1 and -2 to give rise to ET-1 [2,3]. ET-1 is predominantly expressed in endothelial cells to modulate vasoconstriction, neurotransmission, cell proliferation and hormone/cytokine production in autocrine and paracrine fashion [4].

Atherosclerosis, a life-threatening disease, is known to be associated with the excessive proliferation of vascular smooth muscle cells (VSMCs) [5]. It was previously reported that ET-1 predominantly contributed to the development of atherosclerosis by stimulating VSMC proliferation [6-8]. Plasma ET-1 concentration was also found to be significantly higher in patients with hyperlipidemia and atherosclerosis [9-11]. Thus, ET-1 is currently believed to be an important factor for the development and the progression of atherosclerosis.

Alternatively, big ET-1 is cleaved by chymase to generate ET-1 (1-31), a subtype of ET-1, while chymase is predominantly produced in mast cells [12,13]. Interestingly, it was found that big ET-1 production was often enhanced in association with enhanced chymase activation in patients with myocardial infarction [14,15]. These data suggest that ET-1 (1-31) might also play a key role in cardiovascular diseases.

We previously reported that ET-1 (1-31) was capable of promoting VSMC proliferation, and the effect was as potent as that of ET-1 [8]. Our data was recently confirmed by independent researchers [16].

Our current research presented here extends the previous research by asking the next logical question: Does ET-1 (1-31) induces VSMC migration, which is also a key mechanism for the progression of atherosclerosis. In this study, we investigated whether ET-1 (1-31) could induce VSMC migration and compared its effect with that of ET-1. The role of ET receptors in VSMC migration was also examined using cultured rat aortic smooth muscle cells (RASMCs).

2. MATERIALS AND METHODS

2.1 Cell Culture

All animal experiments were conducted according to the “Guide for the Care and Use of Laboratory Animals” published by the US National Institutes of Health (NIH publication No. 85-23, revised 1985) and approved by the ethical committee of the Animal Laboratory of Nara Medical University. Thoracic aortae of male Sprague-Dawley rats (weighing 250-300 g) were excised and immediately immersed in Dulbecco’s Modified Eagle’s Medium (DMEM) containing penicillin (100 U/ml) and streptomycin (100 µg/ml). Connective tissue and adherent fat were removed from the specimens. Then, the isolated specimens of the aorta were cut open, and the endothelium was removed by gently rubbing off the intimal surface using sharp scissors. Denuded aortae were cut into pieces and placed in cell culture dishes, with the intimal face down. DMEM containing 10% fetal bovine serum and penicillin/streptomycin was gently added to the dishes to cover the explants, without disturbing their orientation. VSMCs were allowed to grow from the explants for 7–10 days, and the tissues

were removed using sterilized fine forceps and washed with the culture medium. Once the cells reached confluence, they were harvested by trypsinization and grown in cell culture dishes (passage 1). Cells at early passages (passage 2 to 5) were used in the experiments. More than 90% of isolated cell were confirmed to be VSMCs based on cell morphology and expression of myosin, as described previously [17]. 0.2% Trypan Blue was used to determine the cell viability. As a result, it was found that >98% cells were viable.

2.2 Wound Healing Assay

In order to evaluate cell migration, wound healing assays were conducted. After incubation in serum-free DMEM for 24 h, cells were treated in the absence or presence of either BQ123 or BQ788 for 5 min prior to administration of either ET-1 or ET-1 (1-31). Next we scraped the cell monolayer in a straight line to create a "wound" with a p200 pipet tip. Cells were then placed at 37℃ for 24 h to assess transverse displacement. The images acquired for each sample were further analyzed quantitatively. Cells that had migrated from the border of the non-scraped cells were counted in 10 microscope fields per dish.

2.3 Statistical Analysis

All experiments were performed in triplicate or more, and the values obtained were described as means ± SEM. After performing a 2-way analysis of variance (ANOVA) to determine the significance between the groups, we used a modified t test with Fisher's post-hoc test for intergroup comparison. A P value of <0.05 was considered to be statistically significant.

3. RESULTS

3.1 Increase in RASMC Migration by ET-1 (1-31)

VSMC migration is an important step in the progression of atherosclerosis. While ET-1 is known to stimulate cell migration [18], The exact role that ET-1 (1-31) plays remains unclear [6-8]. In this study, we investigated the effect of ET-1 (1-31) on RASMC migration using wound healing assays. As shown in Fig. 1, ET-1 (1-31) significantly stimulated VSMC migration in a concentration dependent manner. ET-1 (1-31) at 100 nM caused a 1.38-fold increase in VSMC migration whereas ET-1 at the same concentration resulted in a 1.60-fold increase.

3.2 Effect of BQ123 or BQ788 on ET-1 (1-31)-induced RASMC Migration

There are at least two distinct types of ET receptors, ET_A and ET_B receptors [19], the ET_A receptor is known to mediate cell migration under ET-1 stimulation. Here, we examined the effects of a specific ET_A receptor antagonist, BQ123 [20], and a specific ET_B receptor antagonist, BQ788 [21], on the ET-1 (1-31)-induced RASMC migration. As shown in Fig. 2, BQ123, but not BQ788, significantly inhibited the RASMC migration in response to ET-1 (1-31).

4. DISCUSSION

Our study found that ET-1 (1-31) acted on ET_A receptors and stimulated RASMC migration.

In the progression of atherosclerosis, endothelial barriers are initially disrupted by inflammation under several pathological stresses. Foam cell formations underneath endothelial cells precedes the development of fibrous plaques in which the major components are a necrotic core and VSMCs. It is thought that the aberrant responses of VSMCs, including excessive cell proliferation and migration, could be key mechanisms in the progression of atherosclerosis [5].

ET-1 is the most widely expressed ET of the ET family in the cardiovascular system. A major source of ET-1 is thought to be endothelial cells. The release of ET-1 in endothelial cells is sophisticatedly regulated by constitutive and regulated pathways. Because of its importance, the pathophysiological role of ET-1 in atherosclerosis has been intensively studied by several researchers [14,22].

In turn, ET-1 (1-31), a subtype of ET-1, is also likely to be involved in the development of atherosclerosis. The production of ET-1 (1-31) results from the activation of chymase, which is predominantly expressed in mast cells. Chymase is present in the cytoplasmic secretary granules. These granules are expelled out into extracellular spaces when mast cells are stimulated by pathological conditions [12]. In the aortic fatty streak, chymase-expressing mast cells are accumulated at the atherosclerotic regions [23,24].

In ischemic heart diseases and acute coronary syndrome, increases of big ETs were found to be associated with an increase in protease activities of chymase in the heart [14,15]. Thus indicating that ET-1 (1-31) may be involved in the pathological process of heart diseases. Recently, Oka et al. reported that an elevated concentration of circulating ET-1 (1-31) sustained for a longer period after percutaneous transluminal coronary angioplasty, indicating that ET-1 (1-31) might participate in remodeling of blood vessel and the myocardium [16].

To investigate its mechanisms, our group previously examined whether ET-1 (1-31) was capable of stimulating VSMC proliferation in an in-vitro study [8]. The potential mechanism could be accounted for by PKC-dependent ERK1/2 activation [8]. Here, we have shown that ET-1 (1-31) promoted the migratory response of VSMCs in a concentration dependent manner (Fig. 1). These findings suggest that ET-1 (1-31) might be as important as ET-1 in the progression of atherosclerosis via the induction of VSMC migration and proliferation.

ET-1 is released from endothelial cells and interacts with ET receptors expressed on the surface of smooth muscle cells. ET receptors are currently classified into two subtypes, ET_A and ET_B, and belong to G protein-coupled and 7 transmembrane-spanning domain receptors [19]. Russell et al. investigated the distribution of ET receptor subtypes on aorta vascular smooth muscle and revealed that the ET_A receptor is predominantly expressed and that the expression of the ET_B receptor is minor [25].

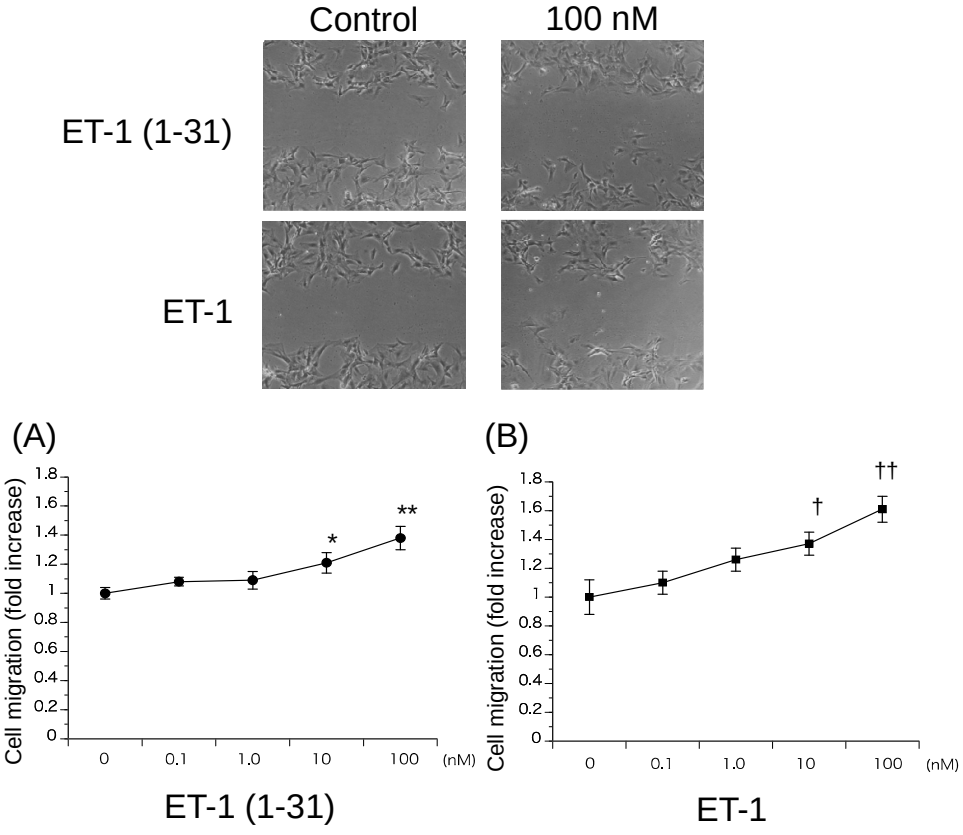

Fig. 1. ET-1 (1-31) induced RASMCs migration with concentration dependent manner

*RASMCs were pre-cultured for 24 h with 1% FBS containing medium. Then, cells were treated with indicated concentration of ET-1 (1-31) (A) or ET-1 (B) and scratched. After 24 h culture, RASMC migration was measured as described under Materials and methods. The asterisks and obelisks represent values that significantly different from the values of ET-1 (1-31) or ET-1 stimulation in normal RASMC (*P=.03, **P=.004, †P=.04, ††P=.006). Each point represents the mean ± SEM (n = 3-4)*

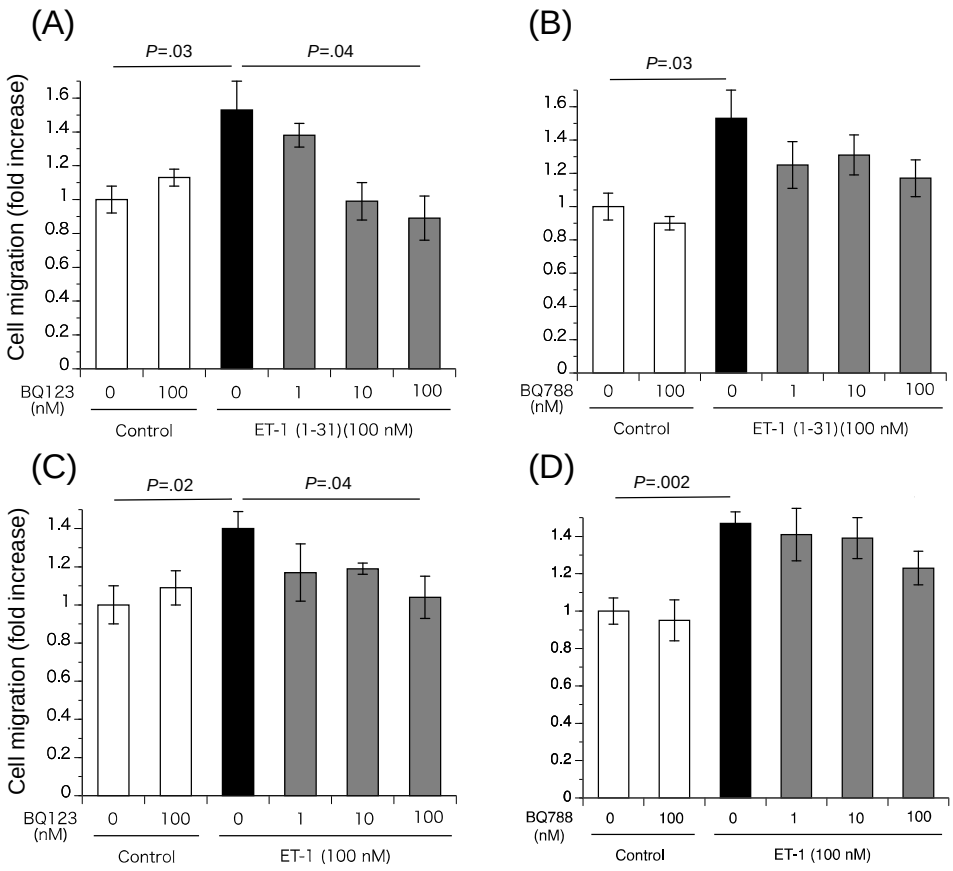

Fig. 2. Inhibitory effects of BQ123 and BQ788 on ET-1 (1-31)-induced RASMC migration

RASMCs were pre-cultured for 24 h with 1% FBS containing medium, and pretreated with indicated concentration of BQ123 (A, C) or BQ788 (B, D) prior to ET-1 (1-31) (A, B) or ET-1 (C, D) treatment. Then, RASMC were cultured for 24 h. RASMC migration was measured as described under Materials and methods. Each point represents the mean ± SEM (n = 3-4)

In this study, we examined which receptors are responsible for the functions of ET-1 (1-31) we observed. We found that BQ123 significantly blocked the ET-1 (1-31)-induced VSMC migration, but BQ788 did not (Fig. 2). Interestingly, this effect was as potent as that of ET-1. The ET_A receptor was found to be a common receptor for ET-1 and ET-1 (1-31), and therefore may play an important role in the progression of atherosclerosis.

Selective ET_A receptor blockers or nonselective ET receptor blockers are currently used in clinical medicine to treat pulmonary arterial hypertension (PAH). Since PAH is characterized by medial hypertrophy due to VSMC proliferation and migration, it is likely that the ET_A receptor is involved in the disease process. As such, using ET receptor blockers might be theoretically suitable for the prophylaxis of atherosclerosis. Nonetheless, there are many factors involved in the progression of arteriosclerosis. Further investigation is warranted.

5. CONCLUSION

In this study, we found that ET-1 (1-31) stimulated VSMC migration. ET-1 (1-31)-induced migration was mediated through ET_A receptors but not ET_B receptors (Fig. 3). Inhibitory effect of a specific ET_A receptor inhibitor BQ123 on ET-1 (1-31)-induced migration is as potent as that on ET-1-induced migration. Because VSMC migration is an important step in the progression of atherosclerosis, our findings suggest that ET-1 (1-31) might play an important role in the progression of atherosclerosis with VSMC migration via ET_A receptor.

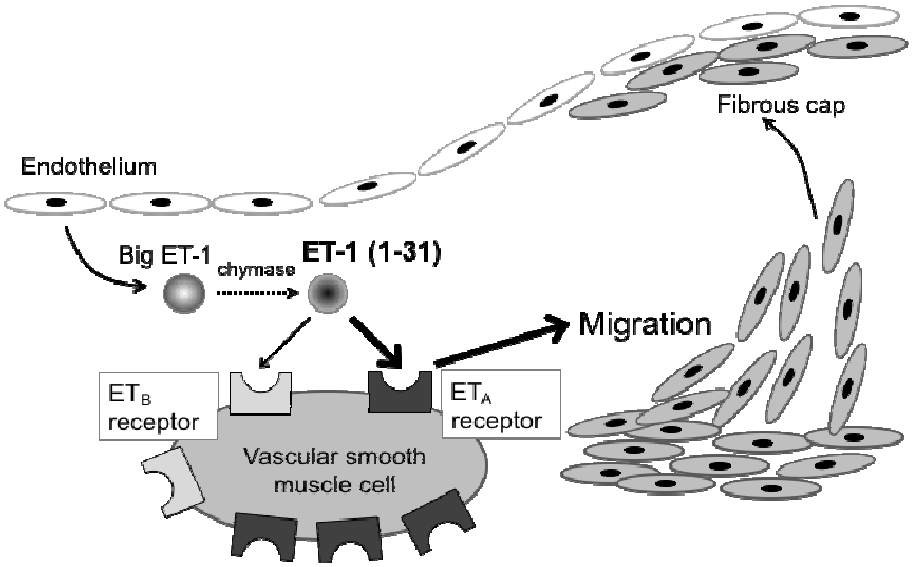

Fig. 3. ET-1 (1-31)-induced migration was mediated through ET_A receptors but not ET_B receptors

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that All experiments were conducted according to the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health (NIH publication No. 85-23, revised 1985) and approved by the ethical committee of the Animal Laboratory of Nara Medical University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Davenport AP, Hyndman KA, Dhaun N, Southan C, Kohan DE, Pollock JS, et al. Pharmacol. Rev. 2016;68:357-418.
2. Shimada K, Takahashi M, Tanzawa K. Cloning and functional expression of endothelin-converting enzyme from rat endothelial cells. J. Biol. Chem. 1994;269: 18275-78.
3. Shimada K, Takahashi M, Ikeda M, Tanzawa K. Identification and characterization of two isoforms of an endothelin-converting enzyme-1. FEBS Lett. 1995;371:140-4.
4. Krämer BK, Nishida M, Kelly RA, Smith TW. Endothelins. Myocardial actions of a new class of cytokines. Circulation. 1992; 85:350-6.
5. Lusis AJ. Atherosclerosis. Nature. 2000; 407:233-41.
6. Chua BH, Krebs CJ, Chua CC, Diglio CA. Endothelin stimulates protein synthesis in smooth muscle cells. Am. J. Physiol. 1992;262:E412-6.
7. Kanse SM, Wijelath E, Kanthou C, Newman P, Kakkar VV. The proliferative responsiveness of human vascular smooth muscle cells to endothelin correlates with endothelin receptor density. Lab. Invest. 1995;72:376-82.
8. Yoshizumi M, Kim S, Kagami S, Hamaguchi A, Tsuchiya K, Houchi H, et al. Effect of endothelin-1 (1-31) on extracellular signal-regulated kinase and proliferation of human coronary artery smooth muscle cells. Br. J. Pharmacol. 1998;125:1019-27.
9. Bath BK, Martin JF. Serum platelet-derived growth factor and endothelin concentrations in human hypercholesterolaemia. J. Intern. Med. 1991;230:313-7.
10. Lerman A, Edwards BS, Hallett JW, Heublein DM, Sandberg SM, Burnett JC Jr. Circulating and tissue endothelin immunoreactivity in advanced

atherosclerosis. N. Engl. J. Med. 1991; 325:997-1001.
11. Stewart DJ, Kubac G, Costello KB, Cernacek P. Increased plasma endothelin-1 in the early hours of acute myocardial infarction. J. Am. Coll. Cardiol. 1991;18:38-43.
12. Nakano A, Kishi F, Minami K, Wakabayashi H, Nakaya Y, Kido H. Selective conversion of big endothelins to tracheal smooth muscle-constricting 31-amino acid-length endothelins by chymase from human mast cells. J. Immunol. 1997;159:1987-92.
13. Fecteau MH, Honoré JC, Plante M, Labonté J, Rae GA, D'Orléans-Juste P. Endothelin-1 (1-31) is an intermediate in the production of endothelin-1 after big endothelin-1 administration *In vivo*. Hypertension. 2005;46:87-92.
14. Miyauchi T, Yanagisawa M, Tomizawa T, Sugishita Y, Suzuki N, Fujino M, et al. Increased plasma concentrations of endothelin-1 and big endothelin-1 in acute myocardial infarction. Lancet. 1989;2:53-4.
15. Urata H, Nishimura H, Ganten D. Mechanisms of angiotensin II formation in humans. Eur. Heart J. 1995;16:79-85.
16. Oka M, Niwa Y, Mawatari K, Hiasa Y, Nakaya Y. A novel peptide of endothelin family, 31 amino-acid length endothelin in patients with acute myocardial infarction. J. Med. Invest. 2014;61:298-305.
17. Kyotani Y, Zhao J, Tomita S, Nakayama H, Isosaki M, Uno M, et al. Olmesartan inhibits angiotensin II-induced migration of vascular smooth muscle cells through Src and mitogen-activated protein kinase pathways. J. Pharmacol. Sci. 2010;113: 161-8.
18. Kohno M, Yokokawa K, Yasunari K, Kano H, Minami M, Yoshikawa J. Effect of the endothelin family of peptides on human coronary artery smooth-muscle cell migration. J. Cardiovasc Pharmacol. 1998;31:S84-9.
19. Davenport AP. International union of pharmacology. XXIX. Update on endothelin receptor nomenclature. Pharmacol. Rev. 2002;54:219-26.
20. Ihara M, Noguchi K, Saeki T, Fukuroda T, Tsuchida S, Kimura S, et al. Biological profiles of highly potent novel endothelin antagonists selective for the ETA receptor. Life Sci. 1992;50:247-55.
21. Ishikawa K, Ihara M, Noguchi K, Mase T, Mino N, Saeki T, et al. Biochemical and pharmacological profile of a potent and selective endothelin B-receptor antagonist, BQ788. Proc. Natl. Acad. Sci. U.S.A. 1994;91:4892-6.
22. Pernow J, Shemyakin A, Böhm F. New perspectives on endothelin-1 in atherosclerosis and diabetes mellitus. Life Sci. 2012;91:507-16.
23. Jeziorska M, McCollum C, Woolley DE. Mast cell distribution, activation, and phenotype in atherosclerotic lesions of human carotid arteries. J. Pathol. 1997; 182:115-22.
24. Lindstedt L, Lee M, Castro GR, Fruchart JC, Kovanen PT. Chymase in exocytosed rat mast cell granules effectively proteolyzes apolipoprotein Al-containing lipoproteins, so reducing the cholesterol efflux-inducing ability of serum and aortic intimal fluid. J. Clin. Invest. 1996;97:2174-82.
25. Russell FD, Skepper JN, Davenport AP. Detection of endothelin receptors in human coronary artery vascular smooth muscle cells but not endothelial cells by using electron microscope auto-radiography. J. Cardiovasc. Pharmacol. 1997;29:820-6.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/16821