Pharmacology of endothelin receptor antagonists ABT-627, ABT-546, A-182086 and A-192621: in vitro studies

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ABSTRACT

Endothelins (ETs), 21-amino-acid peptides involved in the pathogenesis of various diseases, bind to ET\textsubscript{A} and ET\textsubscript{B} receptors to initiate their effects. Based on the same core structure, we have developed four small-molecule ET receptor antagonists, ABT-627, ABT-546, A-182086 and A-192621, which exhibit difference in selectivity for ET\textsubscript{A} and ET\textsubscript{B} receptors. In this report, we compare the potency and selectivity of these four antagonists in inhibiting \( ^{125}\)I-labelled ET-1 binding to cloned human ET\textsubscript{A} and ET\textsubscript{B} receptors, and in blocking ET-1-induced functional responses (arachidonic acid release and phosphatidylinositol hydrolysis).

INTRODUCTION

Endothelin (ET), originally isolated from cultured porcine aortic endothelial cells, is a peptide with 21-amino-acid residues [1]. Three distinct members of the ET family, namely, ET-1, ET-2 and ET-3, have been identified in humans through cloning [2]. Binding of ETs to G-protein-coupled receptors in tissues and cells activates various signalling molecules [3]. Two types of mammalian ET receptors, ET\textsubscript{A} and ET\textsubscript{B}, have been characterized. ET\textsubscript{A} receptor is selective for ET-1 and ET-2, while ET\textsubscript{B} receptor binds ET-1, ET-2 and ET-3 with equal affinity [4–7].

Binding of ETs initiates a complex signal transduction cascade including activation of various kinase-mediated pathways involved in mitogenic responses [8]. ETs affect cell proliferation in various types of cells. Recent evidence suggests that ETs modulate apoptosis induced by serum starvation and chemical treatment, and act as survival factors for fibroblasts, endothelial and smooth muscle cells [9]. ET-1 may play a pivotal role in the pathogenesis of cell growth disorders such as cancer, restenosis, and benign prostatic hyperplasia.

A major advance was made in the ET field with the development of endothelin receptor antagonists [10]. BQ-123 and FR139317 [11–12], two peptidic ET\textsubscript{A}-selective antagonists, are important tools in the investigation of ET-mediated pathophysiology. Following the peptidic compounds, a number of nonpeptide antagonists with improved pharmacokinetics, such as Ro 47-0203 [13], SB 217242 [14], atrasentan [15], etc., were developed. Some of these antagonists are being investigated in human clinical trials [16].

Recent evidence suggests that ET\textsubscript{A} receptor may play a more important pathological role than ET\textsubscript{B} receptor. However, some tissues express ET\textsubscript{B} receptor predominantly, which seems to suggest that ET\textsubscript{B} receptor may also play a pathophysiological role. Thus, ET\textsubscript{A}-selective or ET\textsubscript{B}-selective antagonists could potentially have their unique utilities. Based on the same core
structure, we have developed four nonpeptide ET receptor antagonists, ABT-627, ABT-546, A-182086 and A-192621, which exhibit differences in selectivity for ET\textsubscript{A} and ET\textsubscript{B} receptors. The purpose of this report is to compare the potency and selectivity of these four antagonists in inhibiting [\textsuperscript{125}I]-labelled ET-1 binding to cloned human ET\textsubscript{A} and ET\textsubscript{B} receptors, and in blocking ET-1-induced functional responses.

**MATERIALS AND METHODS**

ET-1 and -3 were purchased from American Peptide Co. (Santa Clara, CA, U.S.A.). [\textsuperscript{125}I]-labelled ET-1 (2200 Ci/mmoll), [\textsuperscript{125}I]-labelled ET-3 (2200 Ci/mmoll), and myo-[\textsuperscript{3}H]inositol (22 Ci/mmoll) were purchased from DuPont New England Nuclear (Boston, MA, U.S.A.). All other reagents were of analytical grade.

**RESULTS**

Figure 1 shows the structures of the four ET receptor antagonists. The potency of ET receptor antagonists on inhibiting ET-1 or -3 binding to ET\textsubscript{A} and ET\textsubscript{B} receptors was evaluated using membranes prepared from CHO cells transfected with the human ET\textsubscript{A} and ET\textsubscript{B} receptor expression constructs. Figure 2 shows the results from competition binding studies for A-192621 and A-182086. In this particular study, the IC\textsubscript{50} values of A-182086 for ET\textsubscript{A} and ET\textsubscript{B} are 0.14 and 0.33 nM respectively. As a comparison, the IC\textsubscript{50} values of A-192621 for ET\textsubscript{A} and ET\textsubscript{B} are 7140 and 5.1 nM respectively. From three independent experiments, the IC\textsubscript{50} values of A-182086 for ET\textsubscript{A} and ET\textsubscript{B} are 0.078 ± 0.021 and 0.284 ± 0.040 nM respectively.

**Cell culture**

Chinese hamster ovary (CHO) cells transfected with the human ET\textsubscript{A} and ET\textsubscript{B} receptor expression constructs, and MMQ cells (a prolactin-secreting clonal cell line) were cultured and maintained as described previously [17].

**Preparation of membranes from tissues and radioligand binding to membranes**

These methods have been described in detail previously [17].

**Phosphatidylinositol hydrolysis**

Evaluation of compounds in antagonizing ET-1-induced phosphatidylinositol hydrolysis in MMQ cells and CHO cells transfected with ET\textsubscript{B} receptor was done according to methods described previously [17].
Antagonists derived from the same core structure can exhibit a diverse range of selectivity towards the two ET receptors. A, arachidonic acid; PI, phosphatidylinositol; ND, not determined. *All values for ABT-627 and ABT-546 have been reported previously [15,17].

<table>
<thead>
<tr>
<th>Study Parameter</th>
<th>Receptor type</th>
<th>ABT-627*</th>
<th>ABT-546*</th>
<th>A-182086</th>
<th>A-192621</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding IC50 (nM)</td>
<td>ET_A</td>
<td>0.055</td>
<td>0.49</td>
<td>0.078</td>
<td>4280</td>
</tr>
<tr>
<td></td>
<td>ET_B</td>
<td>84.8</td>
<td>13000</td>
<td>0.284</td>
<td>8.8</td>
</tr>
<tr>
<td>Ki (nM)</td>
<td>ET_A</td>
<td>0.034</td>
<td>0.46</td>
<td>0.20</td>
<td>5600</td>
</tr>
<tr>
<td></td>
<td>ET_B</td>
<td>63.3</td>
<td>13000</td>
<td>1.23</td>
<td>8.8</td>
</tr>
<tr>
<td>Selectivity</td>
<td>ET_A</td>
<td>1860</td>
<td>28260</td>
<td>6</td>
<td>(836)</td>
</tr>
<tr>
<td>Function AA release</td>
<td>ET_A</td>
<td>0.2</td>
<td>0.59</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>IC50 (nM) PI hydrolysis</td>
<td>ET_A</td>
<td>0.059</td>
<td>3.0</td>
<td>0.05</td>
<td>(\geq 1000)</td>
</tr>
<tr>
<td>IC50 (nM)</td>
<td>ET_B</td>
<td>ND</td>
<td>ND</td>
<td>1.28</td>
<td>0.8</td>
</tr>
</tbody>
</table>

respectively; the IC50 values of A-192621 for ET_A and ET_B are 4280±1610 and 4.54±0.91 nM respectively. Results for ABT-627 and ABT-546 have been previously reported [15,17]. The IC50 values for these four compounds inhibiting 125I-labelled ET-1 binding to ET_A and 125I-labelled ET-3 binding to ET_B are summarized in Table 1. These data indicate that ABT-627 and ABT-546 are ET_A-selective, A-192621 is ET_B-selective, while A-182086 is a non-selective antagonist.

To further examine the nature of the interaction between these compounds and ET receptors, 125I-labelled ET-1 saturation binding studies were performed using membranes prepared from CHO cells stably transfected with the human ET_A and ET_B receptors. Figures 3(A) and 3(C) show the saturation binding curves and their corresponding Scatchard analysis for A-182086 against ET-1 binding to the ET_A receptor. Figures 3(B) and 3(D) show the saturation binding results for A-182086 against ET-1 binding to the ET_B receptor. The K_i values of A-182086 for ET_A and ET_B calculated from this particular study were 0.95 and 2.85 nM respectively. Similar studies were conducted for A-192621. From three independent experiments, the K_i values of A-192086 for ET_A and ET_B are 0.20±0.045 and 1.23±0.130 nM respectively; the K_i values of A-192621 for ET_A and ET_B are 5600±1520

![Figure 3](image-url)
Figure 4 Inhibition of ET-1-evoked phosphatidylinositol hydrolysis

MMQ or CHO cells stably transfected with ET_A were prelabelled with myo-[3H]inositol, and then challenged with 1 nM ET-1 in the presence of increasing concentrations of test compound. Results were calculated by normalizing the increase in signal stimulated by ET-1 in the presence of test agents to control (the increase stimulated by ET-1 in the absence of an antagonist). Data shown are means ± S.D. (n = 3). (A) A-182086, (B) A-192621.

and 8.80 ± 1.51 nM respectively. Results for ABT-627 and ABT-546 have been previously reported [15,17]. The \( K_i \) values of the four compounds are summarized in Table 1. These data indicate that these four compounds decreased the binding affinity of ET-1 without affecting the receptor density, suggesting that they are competitive inhibitors of ET-1 binding. Furthermore, the compounds exhibit different selectivity towards ET_A versus ET_B, which is consistent with the data from the competition binding studies.

The four compounds were also evaluated in functional assays. Figure 4(A) shows that A-182086 effectively inhibited ET-1-evoked phosphatidylinositol hydrolysis in MMQ cells (which contain predominantly ET_A receptor) and in CHO cells stably transfected with the human ET_B receptor with IC_{50} values of 0.06 and 1.12 nM respectively. As a comparison, the IC_{50} values of A-192621 for ET_A and ET_B are 700 and 0.59 nM respectively (Figure 4B). A-182086 and A-192621 alone did not show any agonist or antagonist effects on basal phosphatidylinositol hydrolysis. Results for ABT-627 and ABT-546 in the phosphatidylinositol hydrolysis studies have been previously reported. ABT-627 and ABT-546 have also been tested in an ET-1-induced arachidonic acid release assay mediated by ET_A in human pericardial smooth muscle cells [15,17]. The IC_{50} values of these compounds determined from at least three independent experiments in the functional assays are summarized in Table 1.

DISCUSSION

The development of potent antagonists for ET receptors has aroused keen interest in further developing these agents for clinical utilization [16]. Among the antagonists that exhibit different selectivity towards the two subtypes of ET receptor, it remains a matter of debate and interest as to what kind of antagonists, an ET_A-selective, an ET_B-selective, or a non-selective, may be more clinically beneficial. Emerging evidence suggests that a potent ET_A receptor-selective antagonist may be the choice for therapeutic treatment [18]. However, it is possible that an ET_B-selective or a non-selective antagonist may exhibit specific utility in pathological conditions involving the ET_B receptor.

We have previously reported the development and characterization of A-127722 (ABT-627 or atrasentan) and A-216546 (ABT-546), two novel, nonpeptide ET antagonists that are highly selective for ET_A. In addition, based on the same core structure, we have developed A-192621, an ET_B-selective antagonist, and A-182086, a non-selective antagonist. Data from the receptor binding studies clearly demonstrate that, although the four compounds were developed from the same core structure, they exhibit distinct characteristics in terms of their selectivity towards the two subtypes of ET receptors. Verification of these compounds as functional antagonists was accomplished by inhibition of ET-1-induced phosphatidylinositol hydrolysis and/or arachidonic acid release, and by the lack of any agonist activity of these compounds at concentrations up to 1 \( \mu \)M. Clearly, these four compounds exhibit high potency for the ET receptors. The potency and/or selectivity of atrasentan and ABT-546 for ET_A and that of A-192621 for ET_B are not surpassed by any other known ET receptor antagonist. Furthermore, A-182086 is a truly ‘balanced’ ET_A/ET_B antagonist with very similar \( K_i \) values for the two ET receptor subtypes. More importantly, the compounds share the same core structure, which make them useful for comparing the pathophysiological roles of ET_A versus ET_B independent of the effect of different core structures.

In summary, this paper show that four compounds derived from the same core structure are highly potent
ET receptor antagonists, and exhibit a diverse range of selectivity towards the two ET receptor subtypes.

REFERENCES