Unique receptor dissociation kinetics of the novel endothelin receptor antagonist macitentan

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INTRODUCTION

Pulmonary arterial hypertension (PAH) is caused by progressive pulmonary artery remodeling and contributes to the central pathologic feature of endothelin-1 (ET-1)-induced remodeling in several clinical trials evaluating different ERAs. ET-1 responses are mediated via two G protein-coupled receptors (GPCR) - ET-A and ET-B. In pulmonary arterial smooth muscle cells (PASMC), ET receptors couple to the Gi pathway and increase inositol-triphosphate (IP3) and diacylglycerol (DAG) levels. These cause a biphasic calcium response resulting in sustained elevation of intracellular calcium levels. Elevated calcium levels promote cytoskeletal contraction and cell proliferation (via Rho/ROCK, protease-activated and signal transduction). In normal human PASMC to characterise the inhibitory potency and receptor inhibition kinetics of macitentan versus the approved ERAs bosentan and ambrisentan.

METHODS

Intracellular calcium release measurements using CHO-ET-A and CHO-ET-B cells

Within the fluorescence imaging Plate Reader (FLIPR) Terra Molecular Devices), cells were incubated with Fura2-diol, stimulated with ET-1 and peak relative fluorescence units (RFU) were converted to IP3 values. Kinetic data were calculated via the Cheng-Prusoff equation. The good correlation of antagonistic potencies determined in normal human PASMC and CHO-ET-A cells demonstrated that ET-A receptors are the main contributors to ET-1-induced calcium release in PASMC.

RESULTS

ET-1-induced calcium signalling in PASMC is mainly driven by ET-A receptors

The good correlation of antagonistic potencies determined in normal human PASMC and CHO-ET-A cells demonstrated that ET-A receptors are the main contributors to ET-1-induced calcium release in PASMC.

Intracellular calcium release measurements using normal human PASMC

PASMC (TC5 Cells) were incubated with Fura2-diol and stimulated with ET-1. Peak RFU were converted to IP3 values. Kinetic data were calculated via the Cheng-Prusoff equation (Kb values obtained in PASMC versus CHO-ET-A cells (A) or versus CHO-ET-B cells (B) (Geometric means, n=3–8).

Table 1. Mean Kb values in calcium release kinetic studies using human PASMC with different pre-incubation times (geometric mean, n=3).

<table>
<thead>
<tr>
<th>ERA</th>
<th>0 nM</th>
<th>1 nM</th>
<th>1000 nM</th>
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<tbody>
<tr>
<td>Bosentan</td>
<td>16 nM</td>
<td>1.6 nM</td>
<td>16 nM</td>
</tr>
<tr>
<td>Ambrisentan</td>
<td>14 nM</td>
<td>1.4 nM</td>
<td>14 nM</td>
</tr>
<tr>
<td>Macitentan</td>
<td>1.4 nM</td>
<td>1.4 nM</td>
<td>1.4 nM</td>
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Inositol-1-phosphate (IP1) measurements

PASMC were incubated with ERA dilutions and ET-1 dilutions were added stimulations at 0, 5, 10, 15, 30, 60, 120 min. Calcium release was quantified from FLIPR traces as area under the curve (AUC) between 3 min and 23 min after ET-1 addition. The majority of other ERAs displayed fast apparent association kinetics.

Table 2. ETA & ETB potency and apparent association rates of different ERAs (n=4, FLIPR fluorescence signal, AUC and IC50 values are area under the curve (AUC) between 3 min and 23 min after ET-1 addition).

<table>
<thead>
<tr>
<th>ERA</th>
<th>ETA</th>
<th>ETB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bosentan</td>
<td>1000 nM</td>
<td>1000 nM</td>
</tr>
<tr>
<td>Ambrisentan</td>
<td>1000 nM</td>
<td>1000 nM</td>
</tr>
<tr>
<td>Macitentan</td>
<td>200000 nM</td>
<td>1000 nM</td>
</tr>
</tbody>
</table>

In contrast, macitentan behaved as an insurmountable antagonist.

CONCLUSIONS

Macitentan displays slow receptor dissociation kinetics and a long receptor occupancy half-life in PASMC

Macitentan displays slow receptor dissociation after washout compared to ambrisentan and bosentan. Calculated Rd50 were 17 min (macitentan), 40 sec (ambrisentan) and 30 sec (bosentan).

Macitentan is an insurmountable antagonist of ET-1-induced sustained elevation of intracellular calcium concentrations in PASMC

Bosentan and ambrisentan displayed surmountable antagonism of ET-1-induced sustained elevation of intracellular calcium concentrations in PASMC.

In contrast, macitentan behaved as an insurmountable antagonist.

References


Figure 1. Contribution of endothelin-1 signaling in pulmonary artery smooth muscle cells (PASMC) towards sustained vasoconstriction and vasoconstriction remodelling.

Figure 2. Dissociation kinetics of ambrisentan, bosentan and macitentan determined by calcium release assays in PASMC. Cells pre-incubated with ERA dilutions (120 min) were either directly stimulated with ET-1 (0–60 min) or subjected to washout followed by ET-1 stimulation at different time points. Shown are the time-dependent changes in Kb after washout (geometric mean, n=3±SD from the Kd=1 nM low-turnover, Dunnett’s post test).

Figure 3. ET-1-induced calcium signalling in PASMC is mainly driven by ET-A receptors. Intracellular calcium release measurements using normal human PASMC and CHO-ET-A, or CHO-ET-B pre-incubated with reference ERAs dilutions (120 min) were stimulated with ET-1 (100 nM). Calculation of Kb values obtained in PASMC versus CHO-ET-A cells (A) or versus CHO-ET-B cells (B) (Geometric means, n=3–8).

Figure 4. Dissociation kinetics of ambrisentan, bosentan and macitentan determined by calcium release assays in PASMC. Cells pre-incubated with ERA dilutions (120 min) were either directly stimulated with ET-1 (0–60 min) or subjected to washout followed by ET-1 stimulation at different time points. Shown are the time-dependent changes in Kb after washout (geometric mean, n=3±SD from the Kd=1 nM low-turnover, Dunnett’s post test).

Figure 5. Dissociation kinetics of ambrisentan, bosentan and macitentan determined by calcium release assays in PASMC. Cells pre-incubated with ERA dilutions (120 min) were either directly stimulated with ET-1 (0–60 min) or subjected to washout followed by ET-1 stimulation at different time points. Shown are the time-dependent changes in Kb after washout (geometric mean, n=3±SD from the Kd=1 nM low-turnover, Dunnett’s post test).

Figure 6. The kinetic intracellular calcium response to ET-1 and the effect of bosentan, ambrisentan and macitentan on sustained intracellular calcium elevation (A) ET-1 (log M) measured by Fura2 fluorescence signal recorded in normal human PASMC. B: The time-course of the calcium response following the ET-1 (log M) stimulation and its contribution to the sustained response phase. Representative experiment of n=3.

Figure 7. Effect of bosentan, ambrisentan and macitentan on ET-1-induced calcium accumulation: (A) FLIPR fluorescence signal, (B) Calcium indicator dye Fura2 fluorescence signal recorded in normal human PASMC. B: The time-course of the calcium response following the ET-1 (log M) stimulation and its contribution to the sustained response phase. Representative experiment of n=3.

Figure 8. The kinetic intracellular calcium response to ET-1 and the effect of bosentan, ambrisentan and macitentan on sustained intracellular calcium elevation (A) ET-1 (log M) measured by Fura2 fluorescence signal recorded in normal human PASMC. B: The time-course of the calcium response following the ET-1 (log M) stimulation and its contribution to the sustained response phase. Representative experiment of n=3.