Pharmacology of the Prostaglandin EP₃ Receptor Agonist TEI-3356

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INTRODUCTION

In the last 20 years, our understanding of the pharmacology of arachidonic acid (AA) metabolites has dramatically increased. Prostaglandins (PGs) were first discovered by Ulf van Euler in the 1930s (for review, see ref. 6). PGs are derivatives of C-20 fatty acids (7) and can be synthesized from three related fatty acid precursors, dihomo-γ-linolenic acid (8,11,14-eicosatrienoic acid), arachidonic acid (5,8,11,14-eicosatetraenoic acid), and timnodonic acid (5,8,11,14,17-eicosaantanoic acid). In most species, AA is the precursor of natural prostanoids (for review, see ref. 6). In addition to the two original purified hormones, PGE₁ and PGF₁α, the family of the PGs includes the following members (in alphabetical order): PGA₂ to PGH₂. Then, in the mid-1970s, prostacyclin (PGI₂) (23) and thromboxane A₂ (TXA₂) (10) were discovered.

E-type prostaglandins bind to specific G protein-coupled receptors of the rhodopsin type (6,28,29). Based on their response to various agonists and antagonists (6), these receptors are pharmacologically classified into four distinct subtypes (EP₁ to EP₄). This article reviews the pharmacology of the EP₃ receptor agonist TEI-3356.

CHEMISTRY

TEI-3356, 15-deoxy-16α-hydroxy-16β,20-dimethyl-Δ⁶,⁶α,Δ⁶α-carba PGI₁ (Fig. 1), has a molecular weight of 362. TEI-3356 contains an ω-chain identical to that of misoprostol, a prostaglandin E₁ (PGE₁) analog with potent EP₂ and EP₃ agonistic properties. TEI-3356 has the same potency as an EP₃ receptor agonist as PGE₂ or sulprostone (26). TEI-3356 is soluble in a combination of 5% ethanol, 0.5% Tween 80, and 0.9% saline (e.g., after the
intravenous application, the final concentration in vivo was less than 0.1% for ethanol and 0.01% for Tween 80) (39).

**IN VITRO PHARMACOLOGY**

Radioligand Binding Studies

TEI-3356 inhibits the specific binding of [3H]PGE₂ to EP₃ receptors expressed on Chinese hamster ovary (CHO) cells in the following rank order of potency: TEI-3356 > sulprostone > PGE₂ > misoprostol. The specific binding of [3H]PGE₂ to EP₁ receptors expressed on CHO cells is inhibited by the ligands in the following rank order of potency: PGE₂ = sulprostone > misoprostol > TEI-3356. The specific binding of [3H]PGE₂ to EP₂ receptors (expressed on CHO cells) is inhibited by the ligands in the following rank order of potency: PGE₂ > misoprostol > TEI-3356 ≥ sulprostone (26). TEI-3356 is a weak ligand for the iloprost (IP) receptor and, hence, a unique EP₃ receptor ligand, which contains a prostacyclin-like moiety (Fig. 2).

Effects of TEI-3356 on cAMP Formation

Similar to other EP₃ receptor agonists, such as ONO-AE-248 (38) or M&B 28767 (33), TEI-3356 inhibits the increase in cAMP caused by forskolin in CHO cells expressing the EP₃ receptor. This effect is concentration-dependent (26) and is secondary to inhibition of adenylate cyclase. In this assay, TEI-3356 demonstrates its full agonist activity on the EP₃ receptor subtype, compared with those of sulprostone and misoprostol. However, TEI-3356 has almost the same potency as sulprostone (IC₅₀ for TEI-3356: ~10 pmol; IC₅₀ for sulprostone: ~10 pmol; Fig. 3).
Effects of TEI-3356 on \( G_\text{i} \) Activity

The effects of many hydrophil hormones are secondary to binding and activation of membrane receptors. Ligand-binding sites have been studied in various G-protein-coupled rhodopsin-type receptor models. In 1997, Negishi and colleagues reported that TEI-3356 binds to Arg-309 of the EP\(_3\) receptor through ionic interaction. The same authors have also reported that TEI-3356 leads to \( G_\text{i} \) activation and subsequently to a decrease in cAMP formation in CHO cells (3,27,28).

FIG. 2. Effects of PG ligands on \(^{3}\text{H}\)PGE\(_2\) binding to EP\(_1\), EP\(_2\), and EP\(_3\) and on \(^{3}\text{H}\)iloprost binding to IP. The membrane fractions prepared from the indicated receptor-expressing CHO cells were incubated with either 4 nM \(^{3}\text{H}\)PGE\(_2\) (EP\(_1\), EP\(_2\), or EP\(_3\)) or 20 nM \(^{3}\text{H}\)iloprost (IP) in the presence of various concentrations of TEI-3356 (○), sulprostone (△), misoprostol (□), PGE\(_2\) (○; EP\(_s\)), or iloprost (○; IP). All values were corrected for nonspecific binding and are expressed as percentages of the respective controls, as described under Materials and Methods. Specific binding in the controls was 0.1 (EP\(_1\)), 0.2 (EP\(_2\)), and 0.5 (EP\(_3\)) pmol/mg for \(^{3}\text{H}\)PGE\(_2\) and 0.7 pmol/mg (IP) for \(^{3}\text{H}\)iloprost, respectively. The results shown are the means for three independent experiments, which varied by less than 5%. From ref. 26.
Relaxing Effects of TEI-3356 on Human Pulmonary Artery

TEI-3356 has been suggested to be a highly selective EP₃ receptor agonist (26), although it is a moderate agonist of the EP₁ receptor (EC₉₀ for agonist potency on CHO cells expressing EP₁ receptors: TEI-3356 ~ 100 µmol (26)) and only 100 times less potent than iloprost in competing with [³H]iloprost binding on the prostacyclin (IP) receptor. In preparations of human lung tissue, Jones et al. (18) observed some contractile activity of TEI-3356 in the presence of thromboxane (TP) receptor blockade, suggesting an activation of EP₃ receptors. However, this effect was present only in some preparations and masked by the dominant IP-mediated relaxant activity of TEI-3356. Although the IP-mediated relaxant activity of TEI-3356 is relatively weak when compared with other prostanoids (e.g., cicaprost), the authors still suggest that TEI-3356 may not be specific enough to count as a selective EP₃ receptor agonist (Fig. 4).

Effects of TEI-3356 on Vascular Smooth Muscle Cells

Nakaki et al. (24,25) investigated the effects of prostacyclin and carbacyclins of endothelin-induced DNA synthesis in cultured vascular smooth muscle cells. DNA synthesis

![Graph](image)

**FIG. 3.** Effects of PG ligands on forskolin-induced cAMP formation in EP₃-expressing CHO cells. CHO cells stably expressing EP₃ were incubated for 10 min at 37°C with the indicated concentrations of TEI-3356 (■), sulprostone (△), misoprostol (□), or PGE₂ (○) in the presence of 3 µM forskolin and 1 mM 3-isobutyl-1-methylxanthine. The cAMP content was measured as described under Materials and Methods. The results shown are the means for three independent experiments, which varied by less than 5%. From ref. 26.
was estimated by measuring the incorporation of \(^{3}H\)thymidine into rat aortic smooth muscle cells. TEI-3356, prostacyclin, and other carbacyclins inhibited the endothelin-induced DNA synthesis, suggesting that prostacyclin and its stable analogs may be effective in preventing smooth cell proliferation and possibly the progress of atherosclerosis (24,25). It is unclear whether this antiproliferative effect of TEI-3356 is mediated by activation of IP or EP receptors.

**IN VIVO PHARMACOLOGY**

Effects on Myocardial Ischemia and Reperfusion Injury in Rats

Myocardial ischemia and especially reperfusion of previously ischemic myocardium are associated with an altered formation and release of arachidonic acid (AA) and its metabolites. During myocardial ischemia and reperfusion in vivo, there are a significant

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**FIG. 4.** Prostacyclin analogs (upper) and nonprostanoid prostacyclin mimetics (lower): comparison (means ± S.E.M.) of log IC\(_{50}\) values for relaxation of human pulmonary artery (○, n = 4) and log K\(_i\) values for binding to IP receptor on human platelet membranes (●, n = 3). Benzo-PGI, benzodioxane prostacyclin. The arrow adjacent to the log IC\(_{50}\) for 15-deoxy-16α-hydroxy-16β,20-dimethyl-Δ\(^{6,6α}\)-6α-carba PGI\(_1\) (TEI-3356) indicates that its IP agonist potency may be greater, because EP\(_3\) contraction opposes IP relaxation. For CU 602, the log IC\(_{50}\) relates to three experiments only, because relaxation in a fourth experiment did not achieve 50%. From ref. 18.
release and accumulation of AA due to activation of phospholipase A₂ or phospholipase C (4, 17, 31, 36). In 1973, Hutton et al. (16) reported that systemic application of PGE₁ produces a hemodynamic profile, which is beneficial in myocardial ischemia. Since then, a number of studies have demonstrated that PGE₁ reduces ischemic myocardial injury in different animal models (30). Similarly, PGI₂ reduces the progression of tissue injury in response to coronary artery occlusion (20) in a variety of species (for review, see refs. 19, 34, and 35).

The effects of E-type prostaglandins are mediated by specific G-protein-coupled receptors (EP receptors) that have been classified into four subtypes, EP₁, EP₂, EP₃, and EP₄ (5). Activation of Gₛ protein and secondary stimulation of adenylate cyclase due to EP₂ receptors (5) lead to a reduction in afterload, an increase in coronary blood flow, inhibition of platelet function, and/or inhibition of the activation and extravasation of polymorphonuclear (PMN) cells (21), all of which may be beneficial in experimental myocardial ischemia. In addition, the protection of isolated cells or organs by prostaglandins has been attributed to an ill-defined “cytoprotective” or “cardioprotective” effect of these agents. The exact mechanism(s) underlying or the prostanoid receptor(s) mediating this effect is (are) unknown (32).

We have recently discovered that the cardioprotective effects of E-type prostaglandins are (at least in part) due to activation of EP₃ receptors that, in turn, leads to the activation of protein kinase C (PKC) and opening of ATP-sensitive potassium (K_ATP) channels (37–39). This hypothesis is supported by the following findings: 1) The cardioprotective effects of PGE₁ (nonselective agonist for all EP receptors), sulprostone (selective agonist of EP₁ and EP₃ receptors), and TEI-3356 (selective agonist of EP₃ receptors) are abolished by inhibition of K_ATP channels with glibenclamide or 5-hydroxydecanoate (11, 12, 37–39). 2) Activation of EP₃ receptors may result in activation of protein kinase C (PKC) (5, 6, 37–39). 3) Sulprostone and TEI-3356 cause cardioprotection without having any hemodynamic (EP₂ mediated) effects (12, 37–39). Following the original observation by Hohlfeld and colleagues (13–15) that EP₃ receptors are expressed on cardiomyocytes and are upregulated following ischemia of the heart, we hypothesized that it is the activation of EP₃ receptors that accounts for the “cardioprotective” and/or “cytoprotective” effects of E-type prostaglandins.

Cardioprotective Mechanism

In 1999, we reported that TEI-3356, an agonist of the prostanoid EP₃ receptor, reduces the size of myocardial infarct caused by coronary artery occlusion and reperfusion in the anesthetized rat (39). In this study, the observed reduction in infarct size was approximately 35% and similar to the reduction in infarct size caused by either PGE₁ (nonselective EP₃ receptor agonist) or sulprostone (selective EP₁/EP₃ receptor agonist) in the anesthetized rabbit (11, 12). Although moderate in magnitude, any reduction of infarct size is important for cardiac function and the clinical outcome of patients.

What, then, is the mechanism(s) by which agonists of the EP₃ receptor, for example, TEI-3356, reduce myocardial infarct size? Our studies have clearly demonstrated that a reduction in blood pressure and, hence, myocardial oxygen consumption does not account for the cardioprotective effect of TEI-3356 (39). There is a strong positive correlation between myocardial oxygen consumption and pressure-rate index (1) and, hence, the
FIG. 5. Myocardial ischemia caused by occlusion (25 min) and reperfusion (2 h) of the left anterior descending coronary artery in the anesthetized rat. Different groups of animals were treated with vehicle (control (Con), \( n = 8 \)), TEI-3356 (TEI, 1 \( \mu \)g/kg/h i.v., \( n = 6 \)), 5-hydroxydecanoate (5-HD, 5 mg/kg i.v., \( n = 6 \)), and 5-hydroxydecanoate plus TEI-3356 (\( n = 6 \)).

5a. area at risk (expressed as percentage of left ventricle) in rats subjected to coronary artery occlusion; 5b. infarct size expressed as percentage of the area at risk (AR) caused by occlusion and reperfusion of the left anterior descending coronary artery. \( \ast \), \( P < 0.05 \) when compared with control. From ref. 39.
observed cardioprotective effect of this EP\(_3\) receptor agonist is not likely to be secondary to a reduction in myocardial oxygen demand. Our finding that TEI-3356 did not reduce blood pressure in the rat also confirms that (at the doses used in our study) the observed cardioprotective effects are not due to activation of EP\(_2\) receptors \textit{in vivo}, which would result in stimulation of G\(_s\), an increase in cAMP, and vasodilatation. Thus, it is very unlikely that the dose of TEI-3356 used in our studies is sufficient to activate EP\(_2\) receptors \textit{in vivo}. We, therefore, have proposed (8) that the observed cardioprotective effects are due to the activation of EP\(_3\) receptors. In order to gain a better insight into the mechanism(s) by which EP\(_3\) receptor agonists reduce myocardial infarct size in the rat, we elucidated the signal transduction events underlying the observed cardioprotective effects.

However, in this study, TEI-3356 was administered prior to regional myocardial ischemia and reperfusion, which could be considered to be a pretreatment. The clinical situation is different, as patients with acute myocardial infarction require a reperfusion intervention therapy. Thus, further investigations in which TEI-3356 is administered during reperfusion are warranted.

Role of ATP-sensitive Potassium Channels

The reduction in infarct size caused by either PGE\(_1\) or by the EP\(_1\)/EP\(_3\) receptor agonist sulprostone is (at least in part) due to the activation and opening of K\(_{\text{ATP}}\) channels (11,12). The cardioprotective effect(s) of TEI-3356 (Fig. 5) and other EP\(_3\) receptor agonists such as M&B 28767 is abolished by pretreatment of the animals with 5-HD (37–39), a selective blocker of K\(_{\text{ATP}}\) channels (9). Although glibenclamide is not a specific inhibitor of cardiac K\(_{\text{ATP}}\) channels, both glibenclamide and 5-HD have been used to document that the cardioprotective effects of ischemic preconditioning involve the activation of K\(_{\text{ATP}}\) channels (12). In a previous report, we have confirmed that the reduction in infarct size, afforded by M&B 28767, is also abolished by a dose of glibenclamide, which has previously been shown to abolish the cardioprotective effects of “ischemic preconditioning” (12). These findings suggest that TEI-3356 and presumably the activation of EP\(_3\) receptors lead to the opening of K\(_{\text{ATP}}\) channels, which, in turn, results in cardioprotection. The mechanism by which opening of K\(_{\text{ATP}}\) channels protects the myocardium against ischemic injury is not clear.

However, compounds that open K\(_{\text{ATP}}\) channels, such as bimakalim (22) or nicorandil (2), exert cardioprotective effects in different models of myocardial ischemia/reperfusion. One could argue that directly opening K\(_{\text{ATP}}\) channels may have more advantages than EP\(_3\) receptor activation, as the mechanisms involved are further downstream in the signaling pathway and therefore may produce fewer side effects. Although the infarct size reduction achieved using the K\(_{\text{ATP}}\) channel opener is greater in magnitude than with EP\(_3\) receptor agonists, many investigators have found hemodynamic side effects, such as reduction in arterial blood pressure (2,22). Thus, further studies are required to investigate in more detail the mechanism of action and side effects of both groups of compounds.

Role of Protein Kinase C

The signal pathway events involved in the cardioprotective effect(s) of TEI-3356 are reminiscent of those that mediate the potent antiischemic effects of “ischemic precondi-
FIG. 6. Myocardial ischemia caused by occlusion (25 min) and reperfusion (2 h) of the left anterior descending coronary artery in the anesthetized rat. Different groups of animals were treated with vehicle (control (Con), \( n = 8 \)), TEI-3356 (TEI, 1 \( \mu \text{g/kg/h i.v.} \), \( n = 6 \)), staurosporine (Stau, 1 \( \mu \text{g/kg i.v.} \), \( n = 6 \)), and staurosporine plus TEI-3356 (\( n = 6 \)).

- **a**, area at risk (expressed as percentage of left ventricle) in rats subjected to coronary artery occlusion;
- **b**, infarct size expressed as percentage of the area at risk (AR) caused by occlusion and reperfusion of the left anterior descending coronary artery. *, \( P < 0.05 \) when compared with control. From ref. 39.
Myocardial ischemia caused by occlusion (25 min) and reperfusion (2 h) of the left anterior descending coronary artery in the anesthetized rat. Different groups of animals were treated with vehicle (control (Con), n = 8), TEI-3356 (TEI, 1 μg/kg/h i.v., n = 6), chelerythrine (Chel, 0.7 mg/kg i.v., n = 6), and chelerythrine plus TEI-3356 (n = 5).

7a. Area at risk (expressed as percentage of left ventricle) in rats subjected to coronary artery occlusion; 7b. Infarct size expressed as percentage of the area at risk (AR) caused by occlusion and reperfusion of the left anterior descending coronary artery. *, P < 0.05 when compared with control. From ref. 39.
tioning” (8). There is evidence that “preconditioning of the myocardium” with ischemia leads to the activation of G-protein-coupled receptors, resulting in activation of protein kinase C, opening of KATP channels, and ultimately cardioprotection (8). Having demonstrated that the cardioprotective effect of the EP3 receptor agonist TEI-3356 is abolished by 5-HD, we have investigated the potential role of PKC in the observed cardioprotective effect(s) of this agent. In this study, the reduction in infarct size afforded by TEI-3356 in the rat was abolished by two inhibitors of PKC, namely, staurosporine and chelerythrine (39) (Figs. 6 and 7). These findings suggest that the signal pathway events leading to a reduction in infarct size caused by the EP3 receptor agonist TEI-3356 involve the activation of PKC.

**SUMMARY**

There is good evidence that TEI-3356

1. is a selective EP3 receptor agonist.
2. inhibits the formation of cAMP in CHO cells expressing the EP3 receptor due to Gs activation in a concentration-dependent fashion.
3. inhibits endothelin-induced DNA synthesis in vascular smooth muscle cells.
4. reduces infarct size in rats subjected to myocardial ischemia and reperfusion. The mechanism(s) of the cardioprotective effects of TEI-3356 is not entirely clear but may involve the activation of PKC and the opening of ATP-sensitive potassium channels.

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