J. Pharm. Pharmacol. 1986, 38: 754–757
Received June 4, 1986

Pharmacological actions of ICI 180080, a novel thromboxane receptor antagonist

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ICI 180080 (5(Z)-7-[2,2-dimethyl-4-(2-hydroxyphenyl)-1,3-dioxan-*cis*-5-yl] heptenoic acid) potently inhibited contractions of rat and rabbit aortae and guinea-pig trachea elicited by 11,9-epoxymethano PGH_2 (U-46619). This antagonism was selective because contractions of aortae to noradrenaline and 5-hydroxytryptamine and trachea to histamine were not antagonized by ICI 180080. Schild analysis of data obtained from experiments on rabbit aortae indicated that this thromboxane receptor antagonism was competitive (pA_2 = 7·50, slope = 1·07). Addition of ICI 180080 to human platelet-rich plasma caused dose-related inhibition of U-46619-induced platelet aggregation. This modification of platelet aggregation was not associated with inhibition of thromboxane synthetase, cyclo-oxygenase or lipoxygenase. ICI 180080 did not modify the primary phase of ADP-induced aggregation of human platelets neither did it affect the platelet inhibitory activity of prostacyclin. When dosed orally to anaesthetized guinea-pigs, ICI 180080 (5–50 mg kg^{-1}) caused dose-related inhibition of U-46619-evoked bronchoconstriction. We conclude that ICI 180080 is a potent, selective, competitive, orally active thromboxane antagonist.

Studies of the cyclic pathways of arachidonic acid metabolism led to the discovery that isomerization of prostaglandin H_2 afforded the unstable products thromboxane A_2 (TxA_2) (Hamberg et al 1975) and prostacyclin (PGI_2) (Bunting et al 1976). The potent and opposing pharmacological actions of TxA_2 and PGI_2 on both vascular smooth muscle and platelets prompted the hypothesis (Moncada & Vane 1979) that the balance between these arachidonic acid metabolites contributed to vascular homeostasis. Consequently vascular injury, resulting in impaired local PGI_2 synthesis may result in vasoconstriction and platelet aggregation. One pharmacological method of intervention designed to restore the balance of PGI_2 and TxA_2 activities would be the use of a specific TxA_2 receptor antagonist. Recently a novel series of 1,3-dioxane TxA_2 antagonists has been reported (Brewster et al 1986). This series is typified by ICI 159995 which had the profile of a competitive, selective TxA_2 antagonist at platelet, pulmonary and vascular receptors (Jessup et al 1985). We now describe the pharmacological activity of 5(Z)-7-[2,2-dimethyl-4-(2-hydroxyphenyl)-1,3-dioxan-*cis*-5-yl]heptenoic acid (ICI 180080), a potent, orally active thromboxane antagonist.

MATERIALS AND METHODS

Smooth muscle

Thoracic aortae from Alderley Park Strain rats (male, 250–350 g) and New Zealand White rabbits (male 2.5–3 kg) were spirally cut, immersed in oxygenated Krebs–Henseleit solution containing indomethacin (10^{-5} M) and subjected to resting tensions of 1 and 2·5 g, respectively. Similarly, lengths of ileum, and tracheal chains from Dunkin–Hartley guinea-pigs (male, 300–500 g) were subjected to resting tensions of 0·5 g. Agonsit dose-response curves were obtained using either the cumulative (rat and rabbit thoracic aortae, guinea-pig tracheal chain) or single dose of increasing concentration (guinea-pig ileum) method. pA_2 values were calculated by determining the agonist EC50 in the absence, and 60 min after addition of antagonists to the bathing Krebs–Henseleit solution. The EC50 was defined as the concentration of agonist producing 50% of the maximum control response throughout. Responses were recorded isotonically throughout.

Human platelets

Venous blood was collected from an antecubital vein of human volunteers who were drug-free for at least one week. Blood was collected into 3·2% (w/v) trisodium citrate (1 part to 9 parts whole blood), centrifuged at 200*g* for 10 min and the platelet-rich plasma aggregated in a Payton aggregometer. Platelet count was not adjusted. Aggregation was measured in terms of of extent, and expressed as a percentage of the maximum control response.

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Enzyme inhibition

The method described by Howarth et al (1982) was used to investigate the activity of ICI 180080 on thromboxane synthetase, 12-lipoxygenase and cyclo-oxygenase.

Konzett Rossler

Fasted Dunkin–Hartley guinea-pigs (male, 250–350 g) were anaesthetized with pentobarbitone (60 mg kg^{-1} i.p.). Anaesthesia was maintained with intravenous administration of pentobarbitone (6 mg ml^{-1}) and a tracheal cannula was inserted to permit measurement of air overflow according to the method of Konzett & Rossler (1940). Constant volumes of increasing concentrations of agonist were administered in 0·9% NaCl (saline) via the jugular vein and the resulting dose-related bronchoconstriction measured on a flatbed recorder. A control dose-response curve was obtained to U-46619 in each animal before, and at 30 min intervals after administration of ICI 180080 or vehicle (p.o.). Dose-ratios were calculated at the ED50, defined as the dose of agonist producing 50% of the maximum control response throughout.

Spontaneously breathing guinea-pigs

Fasted, Dunkin–Hartley guinea-pigs (male, 250–350 g) were anaesthetized with alphaxalone (5 mg kg^{-1} i.v.), the jugular vein was cannulated and anaesthesia maintained by constant infusion. The trachea was cannulated and airflow measured by a pneumotachograph and differential pressure transducer. A cannula was inserted into the pleural cavity to permit measurement of pleural pressure. Airway resistance and dynamic compliance were monitored on a breath by breath basis (Amdur & Mead 1958).

The following drugs and chemicals were used: acetylcholine chloride, histamine diphosphate, 5-hydroxytryptamine creatinine sulphate, (−)-noradrenaline bitartrate, (−)-adrenaline bitartrate, adenosine-5′-diphosphate, indomethacin, prostaglandin E_2(PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Sigma Chemical Co.), U-46619 ((15S)-hydroxy-11α,9α-(epoxymethano)-prosta-5Z,13E-dienoic acid, Upjohn Co.), equine collagen fibrils (Hormon-Chemie).

All except the following compounds were prepared as stock solutions in either saline or distilled water. ICI 180080 was dissolved in either dimethyl sulphoxide or 10 mM Na_2CO_3. Collagen was dissolved in a commercially supplied buffer. PGE_2 and U-46619 were dissolved in ethanol (30% wt vol) and (−)-noradrenaline and (−)-adrenaline were solubilized in saline containing 5 × 10^{-3} M ascorbic acid. Further dilutions of the above were made using saline or Krebs–Henseleit, as appropriate.

RESULTS

Smooth muscle

Rabbit isolated thoracic aorta preparations were exposed to increasing concentrations of the TxA_2 mimetic U-46619 in both the absence and presence of ICI 180080. ICI 180080 caused parallel shifts to the right of the U-46619 dose-response curve suggesting competitive antagonism (not shown). Analysis (Arunlakshana & Schild 1959) of the data yielded a pA_2 value (mean ± s.e.m.) of 7·50 ± 0·05 (n = 4) and the slope of the Schild plot (1·07) did not differ significantly from unity ($P > 0·05$).

The specificity of the competitive antagonism observed with ICI 180080 on rabbit aortae was investigated. Rabbit thoracic aortae were exposed to cumulative concentrations of noradrenaline or 5-hydroxytryptamine in the absence and presence of ICI 180080 (2 × 10^{-5} M). The dose-related contractions induced by both agonists were not significantly modified by ICI 180080 (dose ratio < 2, n = 4) (not shown).

The specific receptor antagonism was confirmed using another vascular TxA_2 receptor preparation, the rat thoracic aorta (Coleman et al 1981). When dosed cumulatively both U-46619 and noradrenaline caused dose-related contractions of rat isolated aortae. ICI 180080 significantly blocked U-46619-, but not noradrenaline-induced responses in this preparation (Fig. 1).

Isolated tracheal chain preparations were exposed to cumulative concentrations of U-46619 and histamine in the absence and presence of ICI 180080.

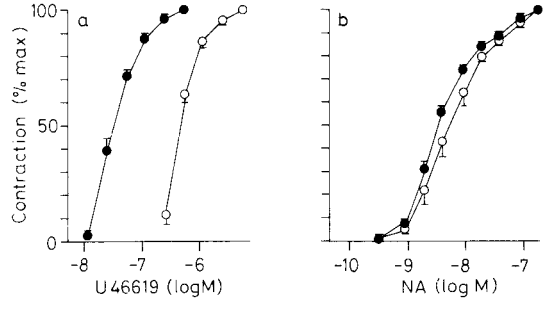

FIG. 1. Response (mean ± s.e.m., n = 4) of rat thoracic aortae to (a) U-46619 in the absence (—●—) and presence of ICI 180080 (1 × 10^{-6} M, —○—) and (b) noradrenaline (NA) in the absence (—●—) and presence of ICI 18080 (1 × 10^{-5} M, —○—).

Both concentrations of ICI 180080 (10^{-7}, 10^{-6} M) caused a parallel shift to the right of the U-46619 dose-response curve suggesting competitive antagonism. The specificity of the antagonist activity in guinea-pig trachea was confirmed by the observation that ICI 180080 (2×10^{-5} M) did not significantly modify histamine-induced contractions of this preparation (Fig. 2).

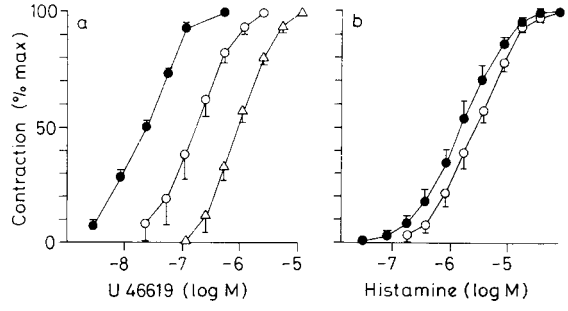

FIG. 2. Response (mean ± s.e.m., n = 4) of guinea-pig trachea to (a) U-46619 in the absence (—●—) and presence of ICI 180080 (1×10^{-7} M, —○—) and (1×10^{-6} M, —△—) and (b) histamine in the absence (—●—) and presence of ICI 180080 (1×10^{-5} M, —○—).

Further investigation of the selectivity of ICI 180080 was carried out on guinea-pig ileum, a preparation preferentially sensitive to prostaglandins of the E series. The concentration-dependent contractions of guinea-pig ileum induced by acetylcholine, histamine, 5-hydroxytryptamine, PGE_2 and $PGF_{2\alpha}$ were not significantly (dose ratio < 2) affected by ICI 180080 (2×10^{-5} M, n = 4) (not shown).

Human platelets

Addition of U-46619 ($3{\cdot}5 \times 10^{-7}$–2×10^{-6} M) to human citrated platelet-rich plasma caused dose-dependent aggregation. When added 60 s before U-46619, ICI 180080 caused parallel shifts to the right of the agonist dose–response curve suggesting competitive antagonism (Fig. 3). Schild analysis yielded a pA_2 value (mean ± s.e.m.) of 6·7 ± 0·04 (n = 4) with a slope (1·028) not differing significantly from unity ($P > 0{\cdot}05$).

Collagen (0·08–4 μg ml^{-1}), when added to human citrated platelet-rich plasma, caused dose-related aggregation. Prior addition of ICI 180080 (5×10^{-7}, 2×10^{-6} and 1×10^{-5} M) to platelet suspensions caused significant inhibition of the collagen response, yielding dose-ratios (mean ± s.e.m.) of 2·51 ± 0·22 (n = 6), 5·41 ± 0·08 (n = 6) and 7·71 ± 2·4 (n = 4), respectively. ICI 180080 (2×10^{-7}–2×10^{-6} M) effectively antagonized the prostanoid-dependent second phase of both adrenaline- and ADP-induced human platelet aggregation (Fig. 4). However, at a final concentration of 1×10^{-4} M ICI 180080 did not significantly modify primary aggregation induced by adrenaline (mean dose ratio ± s.e.m. = 1·2 ± 0·08, n = 4) and ADP (1·04 ± 0·06, n = 4), neither did it effect the platelet inhibitory activity of PGI_2 (not shown). In addition, ICI 180080 (1×10^{-4} M) was devoid of thromboxane synthetase, cyclo-oxygenase and 12-lipoxygenase inhibitory activity in human platelet microsome enzyme preparations (results not presented).

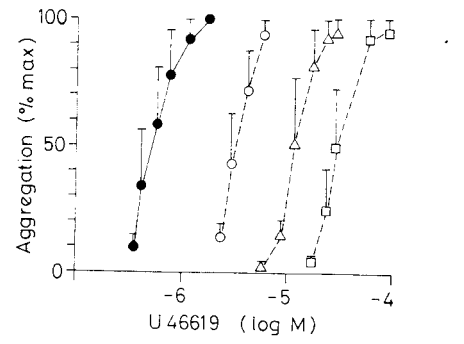

FIG. 3. Aggregation (mean ± s.e.m., n = 4) of human citrated platelets to U-46619 in the absence (—●—) and presence of ICI 180080 (1×10^{-6} M, —○—), (4×10^{-6} M, —△—) and 1×10^{-5} M, —□—).

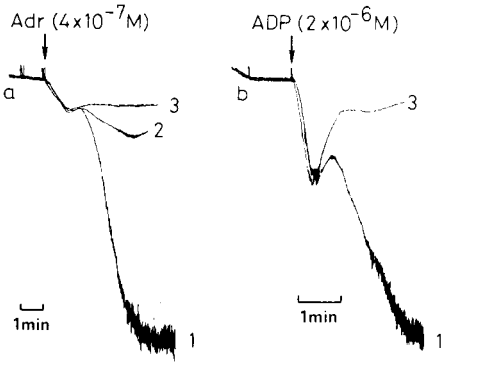

FIG. 4. Typical aggregation responses of human citrated platelets to (a) adrenaline (4×10^{-7} M) and (b) ADP (2×10^{-6} M) in the absence (1) and presence of ICI 180080 (2×10^{-7} M, 2) and (5×10^{-7} M, 3).

Pulmonary preparations

When dosed intravenously to lightly anaesthetized, spontaneously breathing guinea-pigs, ICI 180080 (0·16–40 mg kg^{-1}, n = 4) did not significantly modify

resting values of dynamic compliance and airway resistance (not shown).

U-46619 (0·2–4 μg kg^{-1} i.v.), dosed cumulatively at 30 min intervals, caused reproducible, dose-related bronchospasm when measured by the method of Konzett & Rossler (1940). ICI 180080 (5–50 mg kg^{-1} p.o. in 10 mM Na_2CO_3) caused dose-dependent blockade of subsequent U-46619-induced responses (Fig. 5).

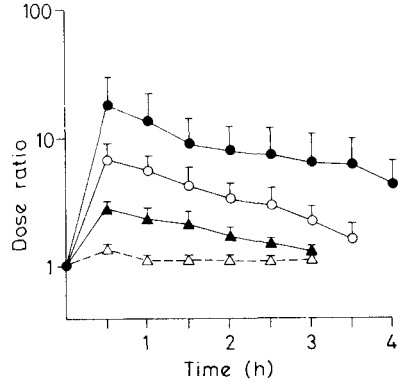

FIG. 5. Bronchoconstriction (mean ± s.e.m., n = 6) induced in anaesthetized guinea-pigs with U-46619 (i.v.) following oral administration of placebo (—△—), ICI 180080 5 mg kg^{-1} (—▲—), 20 mg kg^{-1} (—○—) and 50 mg kg^{-1} (—●—).

DISCUSSION

The endoperoxide analogue U-46619 (11,9-epoxymethano-PGH_2) has been described as a stable TxA_2 mimetic in a variety of smooth muscle systems (Coleman et al 1981). The results of this study show that in-vitro, ICI 180080 caused dose-dependent parallel shifts to the right of U-46619 dose-response curves on rat and rabbit aortae and guinea-pig tracheal chain preparations. This antagonism was shown to be TxA_2 receptor-selective as ICI 180080 did not inhibit contractions of rat and rabbit aortae induced by noradrenaline and 5-HT, neither did it modify contractile responses to histamine on guinea-pig tracheal chain preparations. Furthermore, on guinea-pig ileum, a tissue known to be preferentially sensitive to prostaglandins of the E series, ICI 180080 was devoid of antagonist activity against PGE_2 and a variety of other agonists. The above observations indicate that in-vitro ICI 180080 had the profile of a selective TxA_2 receptor antagonist on vascular and pulmonary smooth muscle.

When human platelets are irreversibly aggregated in-vitro with collagen, ADP and adrenaline, endogenous TxA_2 is generated. Addition of ICI 180080 to human platelet-rich plasma inhibited the TxA_2-dependent phase of collagen, ADP and adrenaline aggregation but did not modify the primary response to adrenaline and ADP. This lack of activity against primary ADP and adrenaline aggregation indicates that ICI 180080 does not significantly stimulate platelet adenylate cyclase (Harris et al 1979). Furthermore ICI 180080 did not modify the platelet inhibitory activity of PGI_2 neither did it significantly inhibit human platelet microsomal thromboxane synthetase, cyclo-oxygenase or lipoxygenase. However, in human platelets ICI 180080 caused dose-dependent parallel shifts to the right of U-46619 aggregation-response curves. The spectra of activities observed with ICI 180080 in these studies characterize this compound as a specific, competitive antagonist at human platelet TxA_2 receptors.

When dosed either orally or intravenously to anaesthetized guinea-pigs, ICI 180080 did not itself modify resting pulmonary parameters but potently inhibited subsequent U-46619-induced changes in respiratory function. Hence oral administration of TxA_2 receptor antagonists may prove an effective anti-bronchospastic therapy.

We have shown that ICI 180080 is a potent and specific receptor antagonist at vascular, pulmonary and platelet TxA_2 receptors which is devoid of TxA_2 synthetase, cyclo-oxygenase or lipoxygenase inhibitory activities. Such an agent may prove beneficial in bronchospastic, vasospastic and thrombotic disorders.

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