0022-3565/93/2673-1017$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 267, No. 3
Printed in U.S.A.

Interactions of Nonprostanoid Trimetoquinol Analogs with Thromboxane A_2/Prostaglandin H_2 Receptors in Human Platelets, Rat Vascular Endothelial Cells and Rat Vascular Smooth Muscle Cells[1]

YANGMEE SHIN[2], KARL J. ROMSTEDT, DUANE D. MILLER[3] and DENNIS R. FELLER

Divisions of Pharmacology (Y.S., K.J.R., D.R.F.), and Medicinal Chemistry and Pharmacognosy (D.D.M.), College of Pharmacy, The Ohio State University, Columbus, Ohio 43210-1291

Accepted for publication August 9, 1993

ABSTRACT

Trimetoquinol (TMQ), a nonprostanoid compound, inhibits thromboxane A_2 agonist-induced responses in platelets and vascular smooth muscle. Sixteen TMQ analogs were used to examine the stereochemical requirements of the interaction with thromboxane A_2/prostaglandin H_2 (TP) receptor sites in human platelets (HP), and cultured rat vascular endothelial (RVEC) and smooth muscle (RVSMC) cells. [^{3}H]SQ 29548 was used as the ligand for TP receptors. The receptor binding affinities of these TMQ analogs for TP receptors in HP, RVEC and RVSMC were highly correlated with each other, and to their reported inhibitory potency values against U46619-induced HP aggregation and serotonin secretion, and contraction of rat aorta. TP receptor binding affinities of TMQ and 8-fluoroTMQ isomers were highly stereoselective (R-isomer > S-isomer), and only the 8-fluoroTMQ isomers gave qualitatively different functional responses in rat aorta. The affinity of TMQ for TP receptors was increased by addition of iodine and fluorine atoms at the 5- and 8-positions of the catechol ring or by replacement of the methoxy groups with iodine atoms on the 1-benzyl ring system. The results indicate that: 1) the stereochemical requirements of TMQ analogs for interaction with TP receptors in these cell systems are the same; 2) although TMQ analogs act as TP receptor antagonists, differences in functional responses by 8-fluoroTMQ isomers in platelets and aorta are not explained by their relative binding affinities to TP receptors; and 3) asymmetric halogenated TMQ analogs should be useful as affinity probes for further characterization of TP receptors.

TXA_2 is one of the most potent naturally occurring activators of platelet aggregation and vasoconstriction leading to a variety of cardiovascular disorders (Halushka and Lefer, 1987; Lefer, 1985) by interacting with its specific receptors. In order to identify TP receptor subtypes, pharmacological and biochemical characterization of the receptors has been studied extensively in platelets (Armstrong *et al.*, 1983; Dorn, 1989, 1991; Hanasaki *et al.*, 1988a, 1989; Hanasaki and Arita, 1988; Hedberg *et al.*, 1988; Kattleman *et al.*, 1986; Mais *et al.*, 1988; Morinelli *et al.*, 1989; Swayne *et al.*, 1988; Takahara *et al.*, 1990), and to a lesser degree in vascular endothelial (Hanasaki *et al.*, 1988b; Hunt *et al.*, 1992; Sung *et al.*, 1989; Swayne *et al.*, 1988) and smooth muscle cells (Hanasaki *et al.*, 1989; Hanasaki and Arita, 1989; Morinelli *et al.*, 1989, 1990).

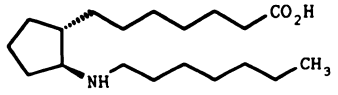
Early functional studies (LeDuc *et al.*, 1981; Lefer *et al.*, 1980) indicated that TP-mediated responses in platelets and vascular tissues differ. Subsequently, Mais *et al.* (1985a,b) suggested that there are receptor subtypes for TXA_2 in platelets and vascular tissue based on differences in the rank order of functional inhibition by TP-receptor antagonists. Radioligand binding studies have revealed a similarity in binding specificity of structurally diverse TP receptor agonists and antagonists in platelets and vascular tissues (Hanasaki *et al.*, 1988a,b; Mihara *et al.*, 1989). However, Masuda *et al.* (1991) reported that a series of 13-azapinane TXA_2 analogs differentiated between TP receptor sites in platelets and vascular smooth muscles.

Received for publication February 12, 1993.
[1] The authors wish to thank the USPHS (HL-22533) from the National Institutes of Health for the support of this work.
[2] Current address: National Institutes of Health, Laboratory of Bioorganic Chemistry, Building 8, Room A-20, Bethesda, MD 20892.
[3] Current address: Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee, 847 Monroe Avenue, Memphis, TN 38163-2111.
[4] Dennis R. Feller, Ph.D., Division of Pharmacology, College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210-1291.

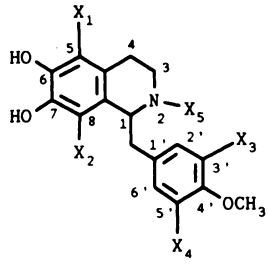
ABBREVIATIONS: TXA_2, thromboxane A_2; TP, thromboxane A_2/prostaglandin H_2; TMQ, trimetoquinol; 5F-TMQ, 5-fluoroTMQ; 8F-TMQ, 8-fluoroTMQ; U46619, (15S-hydroxy-11α,9α-epoxymethanoprosta-5Z,13E-dienoic acid) and SQ 29548, (1S-[1α, 2;β(5Z), 3β, 4a])-7-[3-[[2-[(phenylamino)carbonyl]-hydrazino]methyl)-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid; EGTA, ethylene glycol bis N, N'-tetraacetic acid; PGE, prostaglandin E_1; FCS, fetal calf serum; trans-APA, trans-13-azaprostanoic acid; HP, human platelets; RVEC, rat vascular endothelial cells; RVSMC, rat vascular smooth muscle cells.

Several studies (Dorn, 1991; Furci *et al.*, 1991; Kattleman *et al.*, 1986; Hanasaki *et al.*, 1988b, 1989; Masuda *et al.*, 1991; Mihara *et al.*, 1989) have examined the binding characteristics of prostanoid compounds in platelets and vascular smooth muscle cells. Only a few nonprostanoid TP receptor-interacting compounds were included in those studies. Previous reports from our laboratory (Ahn *et al.*, 1988; Mayo *et al.*, 1981; Mukhopadhyay *et al.*, 1985; Shin *et al.*, 1991, 1992) demonstrated that the nonprostanoid compound, TMQ (fig. 1), was a highly stereoselective (R-isomer ≫ S-isomer), competitive antagonist of TP receptors in human platelets and rat aortae. In recent work, we found that the fluorine-substituted TMQ analogs, 5F-TMQ and 8F-TMQ (fig. 1) are 16- and 29-fold, respectively more potent than TMQ as antagonists of U46619-induced contraction of rat aorta versus human platelet aggregation (Miller *et al.*, 1988); and the TP receptor binding of these TMQ analogs was the same in human and rat platelets (Romstedt *et al.*, 1992). Accordingly, these results imply that the observed functional selectivities of 5F-TMQ and 8F-TMQ are attributed to differences in their TP receptor affinities in vascular smooth muscle and platelets. TMQ, unlike other prostanoid TP antagonists, also shows interspecies selectivity for TP-mediated functional responses in vascular tissues (Ogletree and Allen, 1992). To date, studies of TMQ analog binding interactions have been limited to platelet TP receptors (Ahn *et al.*, 1988; Romstedt *et al.*, 1992; Shin *et al.*, 1991, 1992).

The main objective is to establish whether previously observed tissue and species-selective differences in functional inhibition of TP-mediated responses by nonprostanoid TMQ analogs are explained by their TP receptor affinities in appropriate cell models. The present work characterizes the receptor binding potencies of TMQ analogs for interaction with TP receptors on different cardiovascular cell types (blood platelets, vascular endothelial and smooth muscle cells). Rat aortic smooth muscle, rat aortic endothelial cells and human umbilical venous endothelial cells were selected as representative tissues (Hanasaki *et al.*, 1988a,b) to assess possible intertissue and interspecies differences in TMQ analog interactions on TP receptors. Receptor interactions of these nonprostanoid compounds were studied in these cells using a highly specific TP receptor antagonist, [^{3}H]SQ 29548 (Hedberg *et al.*, 1988).



Trans-13-azaprostanoic acid [trans-APA]



Abbreviations	X_1	X_2	X_3	X_4	X_5
TMQ	H	H	OCH_3	OCH_3	H
5I-TMQ	I	H	OCH_3	OCH_3	H
8I-TMQ	H	I	OCH_3	OCH_3	H
5F-TMQ	F	H	OCH_3	OCH_3	H
8F-TMQ	H	F	OCH_3	OCH_3	H
5,8-diF-TMQ	F	F	OCH_3	OCH_3	H
3'I-TMQ	H	H	I	OCH_3	H
3',5'-diI-TMQ	H	H	I	I	H
NO_2-TMQ	H	H	NO_2	OCH_3	H
NH_2-TMQ	H	H	NH_2	OCH_3	H
NB-NO_2-TMQ	H	H	OCH_3	OCH_3	N-(4-NO_2benzyl)
NB-NH_2-TMQ	H	H	OCH_3	OCH_3	N-(4-NH_2benzyl)

Fig. 1. Chemical structures and abbreviations of trimetoquinol (TMQ) analogs and trans-13-azaprostanoic acid. The asymmetric carbon atom is indicated by the asterisk.

We previously reported (Romstedt *et al.*, 1992; Shin *et al.*, 1991) TP receptor binding and antagonist activities for a limited number of TMQ compounds in human platelets. The present work extends these studies and, for the first time, examines the TP receptor binding affinities of a large series of structurally related TMQ analogs (fig. 1) in other cardiovascular model cell systems (rat vascular smooth muscle and endothelial cells). Analogs of TMQ that contained 1-benzyl group and N- substitutions were chosen because they possessed functional TP antagonist activities that were more and less potent than TMQ, respectively (Fedyna *et al.*, 1987; Harrold *et al.*, 1987, 1988; Shams *et al.*, 1990). Other analogs that exhibited functional (6,7-dihydroxy ring substitutions) (Miller *et al.*, 1988; Markovich *et al.*, 1992) or optical selectivities (isomers of 8F-TMQ and TMQ) (Ahn *et al.*, 1988; Shin *et al.*, 1992, Markovich *et al.*, 1992) for antagonism of TP-dependent responses in aortae and platelets were also included.

Materials and Methods

Materials. [5,6-^{3}H]SQ 29548 (30.0 Ci/mmol) was purchased from Dupont-New England Nuclear (Boston, MA). Trizma HCl, Trizma base, EGTA, EDTA, PGE_1, heat-inactivated (FCS), antibiotic/antimycotic solution, trypsin-EDTA (5 mg/ml trypsin and 2 mg/ml EDTA), Hanks' balanced salt solution (with and without Ca^{++}/Mg^{++}), Masson's trichrome staining agents, bovine serum albumin (Fraction V) and endothelial cell growth supplement were purchased from Sigma Chemical Co. (St. Louis, MO). Type I collagen (Vitrogen 100) was purchased from Collagen Corporation (Palo Alto, CA). Collagenase type IV was obtained from Worthington Biochemical Corp. (Freehold, NJ). U46619 was a gift from the UpJohn Company (Kalamazoo, MI). Dulbecco's modified Eagles medium (DMEM) and RPMI 1640 were obtained from the Cell Culture Service of The Ohio State University. Rabbit antihuman factor VIII serum, nonimmune rabbit IgG serum and fluorescein-conjugated swine antirabbit IgG serum were purchased from DAKO Corp. (Carpinteria, CA). Scintiverse E was obtained from Fisher Scientific (Pittsburgh, PA). Stereoisomers of TMQ [R(+)-TMQ, $[\alpha_D]$ = +24.1, and S(−)-TMQ, $[\alpha_D]$ = −26.9] were gifts from Dr. Yoshio Iwasawa (Tanabe Seiyaku Company, Limited, Saitama, Japan). Trans-APA and the remaining TMQ analogs were prepared in our laboratory, and other chemicals were of reagent grade. Unless indicated, TMQ analogs were used as racemates. Isomers of 8F-TMQ [S(+)-8F-TMQ, $[\alpha_D]$ = +4.4 and R(−)-8F-TMQ, $[\alpha_D]$ = −4.2] were prepared in our laboratory. Chemical structures of trans-APA and TMQ analogs are given in figure 1. TMQ analogs used and abbreviations are as follows: racemate, R(+)- and S(−)-isomers of trimetoquinol [TMQ, R(+)-TMQ and S(−)-TMQ]; racemate, R(−)- and S(+)-isomers of 8-fluoroTMQ [8F-TMQ, R(−)-8F-TMQ and S(+)-8F-TMQ]; 5,8-difluoroTMQ (5,8-diI-TMQ); 5-fluoroTMQ (5F-TMQ); 8-iodoTMQ (8I-TMQ); 5-iodoTMQ (5I-TMQ); 2-(4-nitrobenzyl)TMQ (NB-NO_2-TMQ); 2-(4-aminobenzyl)TMQ (NB-NH_2-TMQ); 3'-aminoTMQ (NH_2-TMQ); 3'-nitroTMQ (NO_2-TMQ); 3'-iodoTMQ (3'I-TMQ); and 3',5'-diiodoTMQ (3',5'-diI-TMQ).

Preparation of washed human platelets. Washed platelets were obtained from platelet-rich plasma (PRP) by a modification of the method of Hedberg *et al.* (1988). PRP was prepared from the blood containing anticoagulant by centrifugation at 200 × *g* for 15 min. A sedimented platelet pellet, obtained from centrifugation of PRP at 1000

× g for 4 min, was resuspended in 50 mM Tris-saline (7.02 g/l Trizma HCl, 0.67 g/l Trizma base, and 9 g/l NaCl, pH 7.2) containing 5 mM EGTA and 1 μM PGE_1, and recentrifuged at 1000 × g for 4 min. The washing procedure was repeated 3 times in the presence of 1 μM PGE_1 to promote uniform suspension. Final suspensions were 1×10^9 intact platelets/ml in 50 mM Tris-saline.

Cultures of rat aortic vascular and human umbilical venous endothelial cells. Primary cultures of endothelial cells from rat thoracic aorta of male Sprague-Dawley rats weighing 250 to 350 g were prepared on cold collagen gels according to the method of McGuire and Orkin (1987). The cells were cultivated in complete media (RPMI 1640 + 20% FCS + 50 μg/ml ECGS + 200 mM glutamine + antibiotics) and subcultured with 0.05% trypsin/0.02% EDTA. Cells between 5th and 8th passages were used for the binding experiments. Endothelial cells were identified using immunofluorescence with factor VIII antigen according to the method of Cole *et al.* (1986).

Human umbilical venous endothelial cells were provided by The Ohio State Cell Culture Service facility. Umbilical veins were rinsed and incubated with 0.1% collagenase in cold phosphate buffered saline (pH = 7.4) for 20 min, and the veins were massaged to remove endothelial cells. Cells were isolated by centrifugation at 900 × g for 10 min, resuspended into 20% FCS, and seeded in culture dishes coated with human fibronectin (5 $\mu g/cm^3$) and passaged using 0.05% trypsin/0.02% EDTA. Confluent cells between the 5th and 8th passages were used for the experiments.

Cultures of rat aortic vascular smooth muscle cells. Rat vascular smooth muscle cells were prepared from explants of the thoracic aorta of 250 to 350 g male Sprague-Dawley rats by the modification of the method of Ross (1971). The explants were incubated with DMEM supplemented with 20% FCS and antibiotics at 37°C in an air-flow chamber. After serial subculture with a 0.1% trypsin/0.04% EDTA solution, cells were harvested between the 5th and 12th passages. Typical 'hill' and 'valley' morphology at confluence were the characteristics of the vascular smooth muscle cells. Light microscopy with Masson's trichrome stain also revealed the syncytial network of ribbon-like cells, slender nuclei and multiple small nucleoli, and longitudinal cytoplasmic striations (Pollak and Kasai, 1964).

Radioligand binding experiments. Human platelet binding experiments were conducted essentially as described by Hedberg *et al.* (1988). Platelets (1×10^8) were incubated with 0.5 nM [^{3}H]SQ 29548 in the absence or presence of 50 μM unlabeled SQ 29548 in Tris-saline buffer with a final volume of 0.5 mL. Inhibition of specific [^{3}H]SQ 29548 binding to washed platelets by TMQ isomers was determined using different concentrations of the drugs or trans-APA as a positive control. Samples were filtered rapidly by vacuum through Whatman GF/B glass fiber filters on a model 12R ucell harvester (Brandel Gaithersburg, MD) and washed for 10 sec with ice-cold Tris saline. The filters were dried for 5 min, placed in plastic vials containing 10 ml of an emulsion-type scintillation fluid (Scintiverse E), and the amount of radioactivity was measured by liquid scintillation spectrometry. Specific binding and net activity (mean ± S.E.) in platelets were 86.3 ± 1.9% and 2265 ± 375 cpm (n = 36). The K_d and B_{max} (fmol/10^6 platelets) were 3.1 and 0.49, respectively, and they gave a Hill coefficient of 1.2 (Shin *et al.*, 1992).

Binding assays for vascular human and rat endothelial and smooth muscle cells were conducted by a modification of Hanasaki *et al.* (1988a,b). Trypsinized cells (1.5 to 2×10^7 cells/ml) were incubated with 5 nM [^{3}H]SQ 29548 in the absence or presence of 10 μM unlabeled SQ 29548 in 50 mM Tris-saline buffer containing 0.1% bovine serum albumin (pH 7.2). Inhibition of specific [^{3}H]SQ 29548 binding to the cells by TMQ analogs was determined using different concentrations of the drugs. The rest of the procedures was the same as in human platelets. The specific binding and net activity (mean ± S.E.) were 66.1 ± 1.2% and 72 ± 2.2%, and 1683 ± 115 cpm and 727 ± 73 cpm in rat vascular smooth muscle cells (n = 31) and endothelial cells (n = 24), respectively. Hill coefficients were equal to 1.0 in both cell systems. The K_d and B_{max} (fmol/10^6 cells) values for SQ 29548 in rat vascular smooth muscle and endothelial cells were 2.9 and 1.6, and 1.8 and 2.1, respectively (Shin *et al.*, 1992).

Data analysis. Experimentally determined K_d and B_{max} values, and Hill coefficients for SQ 29548, and the dissociation constants (K_i) for competing drugs were calculated using the LIGAND computer program (McPherson, 1985) and the equation of Cheng and Prusoff (1973). Comparisons of functional and binding data in different cells and tissues were analyzed by linear regression. Regression data were expressed as slope (s), correlation coefficient (r) and level of significance (P value) for the correlation.

Results

A series of TMQ analogs was selected to examine the chemical specificity for binding to TP receptor sites in these cell systems (fig. 1; see also "Materials and Methods"). In preliminary experiments with human umbilical venous endothelial cells (n = 3), the specific binding of [^{3}H]SQ 29548 (0.1–8.0 nM) did not exceed 10% of the total binding, whereas 67 to 85% specific binding was observed in rat vascular endothelial cells by SQ 29548 over the concentration range of 0.1 to 8.0 nM. Because of the small extent of binding, no further studies were conducted with TMQ analogs as competing drugs in human umbilical venous endothelial cells.

The binding of [^{3}H]SQ 29548 to washed human platelets, rat vascular smooth muscle cells and rat vascular endothelial cells was displaced by nonprostanoid TMQ analogs (15–16 compounds) and trans-APA in a concentration-dependent manner (fig. 2, table 1). The prototypical prostanoid TP receptor antagonist, trans-APA, gave similar K_i values (parentheses) in human platelets (26.4 μM), rat vascular smooth muscle cells (4.0 μM) and rat vascular endothelial cells (12.7 μM) (table 1). As shown in figure 2, the rank order of receptor affinity for a series of 1-benzyl group-substituted TMQ analogs was the same in each of the cell systems [3′, 5′-diI-TMQ > 3′I-TMQ ≥ TMQ > NO_2-TMQ > NH_2-TMQ].

From a structure-activity evaluation of the data given in table 1, greater receptor binding affinities were observed for those TMQ analogs possessing halogen or nitro group substitution on the 1-benzyl group (3′I-TMQ, 3′, 5′-diI-TMQ and NO_2-TMQ), those analogs with halogen substitution on the catechol ring (5F-TMQ ≥ 8F-TMQ > 8I-TMQ > 5I-TMQ), and TMQ and 8F-TMQ isomers of the R-absolute configuration. In each cell system, R-TMQ and R-8F-TMQ were 564- to 933-fold and 7- to 35-fold, respectively, more potent than the corresponding S-isomers. In contrast, 3′-amino (NH_2-TMQ) and N- (NB-NO_2-TMQ and NB-NH_2-TMQ) substituted TMQ analogs were 16- to 105-fold less potent than TMQ as inhibitors of specific [^{3}H]SQ 29548 binding in these receptor systems.

The rank orders and affinity values (pK_i) against the specific [^{3}H]SQ 29548 binding of TMQ analogs were highly correlated between human platelets and rat vascular endothelial cells (fig. 3, upper panel; s = 0.86, r = 0.95, $P < .001$), human platelets and rat vascular smooth muscle cells (fig. 3, middle panel; s = 0.75, r = 0.89, $P < .001$), and rat vascular endothelial cells and rat vascular smooth muscle cells (fig. 3, lower panel; s = 0.88, r = 0.95, $P < .001$).

The inhibitory potency values (pIC_{50} or pK_b) of the TMQ analogs against U46619 (a TXA_2-agonist)-induced human platelet activation and vasoconstriction in rat thoracic aorta (Mukhopadhyay *et al.*, 1985; Harrold *et al.*, 1988; Shams *et al.*, 1990; Romstedt *et al.*, 1992) were highly correlated with their affinity values for the inhibition of specific [^{3}H]SQ 29548

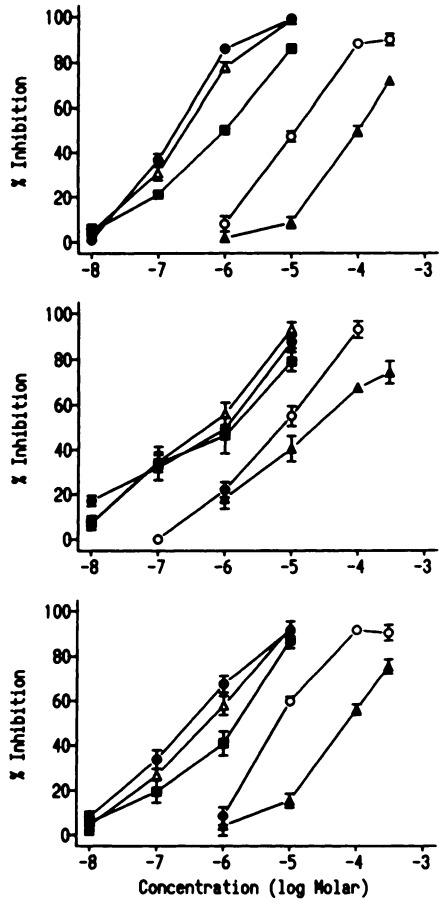


Fig. 2. Competitive inhibition of specific [^{3}H]SQ 29548 binding to washed human platelets (upper panel), rat vascular smooth muscle cells (middle panel) and rat vascular endothelial cells (lower panel) by selected TMQ analogs. Data are presented as the mean ± S.E. of n = 7 to 14 from three different preparations. Key: ● = 3′,5′-dil-TMQ; △ = 3′I-TMQ; ■ = racemic-TMQ; ○ = NO_2-TMQ; and ▲ = NH_2-TMQ (see fig. 1 for structures and abbreviations).

binding in washed human platelets (fig. 4, upper panel; s = 0.74, r = 0.89, P < .001 for aggregation; and fig. 4, middle panel; s = 0.70, r = 0.88, P < .001 for serotonin secretion) and cultured rat aortic vascular smooth muscle cells (fig. 4, lower panel; s = 0.81, r = 0.83, P < .001), respectively.

Discussion

Previous reports have indicated that TMQ: 1) does (Ahn *et al.*, 1988) or does not (Armstrong *et al.*, 1983) interact with TP receptor sites in human platelets; 2) acts at multiple inhibitory sites in human platelets (Ahn *et al.*, 1988); and 3) acts in a qualitatively different manner from other TP receptor antagonists in smooth muscle tissues (Ogletree and Allen, 1992). We have reported recently that TMQ isomers and 1-benzyl group-substituted TMQ analogs inhibit specific [^{3}H]SQ 29548 binding in a concentration-dependent and highly stereoselective manner in human platelets (Shin *et al.*, 1991, 1992). To further investigate the specificity of TMQ analog interactions with TP receptors, we have utilized human platelets, cultured rat aortic vascular endothelial cells and smooth muscle cells as representative TP receptor systems. The TP receptor antagonist, [^{3}H]SQ 29548, bound only to a single class of saturable, high affinity receptor sites in these tissues (Shin *et al.*, 1992), as

TABLE 1

Comparison of the K_i values for inhibition of specific [^{3}H]SQ 29548 binding to human platelets (HP), rat vascular endothelial cells (RVEC) and rat vascular smooth muscle cells (RVSMC)[a]

Compound Tested[b]	K_i (μM)[c]		
	HP	RVEC	RVSMC
trans-APA	26.4 ± 8.8	12.7 ± 2.1	4.03 ± 0.86
TMQ	0.98 ± 0.08	0.41 ± 0.10	0.46 ± 0.13
R(+)-TMQ	0.36 ± 0.05	0.28 ± 0.05	0.26 ± 0.08
S(−)-TMQ	336 ± 57	158 ± 16	233 ± 38
3′I-TMQ	0.21 ± 0.04	0.21 ± 0.06	0.25 ± 0.06
3′,5′-dil-TMQ	0.12 ± 0.02	0.089 ± 0.022	0.38 ± 0.07
NH_2-TMQ	105 ± 7.8	24.0 ± 2.9	9.69 ± 2.54
NO_2-TMQ	8.37 ± 1.11	1.52 ± 0.18	2.63 ± 0.43
NB-NH_2-TMQ	70.5 ± 5.2	8.02 ± 1.52	7.34 ± 2.60
NB-NO_2-TMQ	68.2 ± 8.8	16.5 ± 2.2	23.9 ± 3.7
5F-TMQ	10.8 ± 2.6	10.4 ± 5.5	nd[d]
8F-TMQ	3.82 ± 0.85	2.43 ± 0.64	4.59 ± 1.08
R(−)-8F-TMQ	1.47 ± 0.36	2.38 ± 0.73	3.77 ± 1.11
S(+)-8F-TMQ	51.9 ± 7.5	16.3 ± 2.4	40.6 ± 9.8
5,8-diF-TMQ	35.4 ± 6.5	53.2 ± 12.6	45.3 ± 8.8
5I-TMQ	35.9 ± 8.1	21.8 ± 2.82	8.29 ± 2.37
8I-TMQ	14.7 ± 3.2	8.67 ± 1.84	9.46 ± 2.08

[a] Data represent the mean ± S.E. of 7 to 31 experiments from at least three different cell preparations.
[b] Abbreviations and structures of TMQ analogs are given in figure 1.
[c] K_i values were calculated from the equation of Cheng and Prusoff (1973).
[d] nd = not determined.

previously reported (Hanasaki *et al.*, 1988a,b; Hedberg *et al.*, 1988).

All TMQ analogs were concentration-dependent inhibitors of the specific binding of [^{3}H]SQ 29548 with similar potencies in human platelets and cultured rat vascular endothelial and smooth muscle cells. Furthermore, inhibitory potencies of these compounds in platelets, vascular endothelial cells and smooth muscle cells were highly correlated (fig. 3). Thus, the ligand binding site recognized by TMQ analogs and SQ 29548 is similar in these TP systems.

Heterogeneity of TP receptor populations has been based on pharmacological differences in the antagonist activity of prostanoids (Mais *et al.*, 1985a,b; Tymkewycz *et al.*, 1991). A goal of these studies was to determine whether functional effects of TMQ analogs against TXA_2-agonist-induced responses correlated favorably with TP receptor affinities in platelets and vascular smooth muscle. The present work demonstrates that TMQ analogs bind to TP receptor sites in platelets and vascular smooth muscle cells with similar rank orders of potencies (fig. 3). The binding affinities of TMQ analogs also were highly correlated to previously reported inhibitions of TP-mediated pharmacological responses in platelets and rat aorta (fig. 4). Collectively, the results of our studies with nonprostanoid TMQ analogs are consistent with the previous reports that the TP receptors have similar recognition sites for prostanoid and nonprostanoid analogs in platelets, vascular endothelial and vascular smooth muscle cells (Hanasaki *et al.*, 1988a,b; Huzoor-Akbar *et al.*, 1985; Swayne *et al.*, 1988).

The functional effects of halogenated TMQ analogs, previously reported to exhibit tissue selectivity (Miller *et al.* 1988; Markovich *et al.* 1992), did not correlate to TP receptor affinities. In this regard, 5F-TMQ and 8F-TMQ are selective antagonists of U46619-induced responses in rat aorta versus human platelets in comparison to TMQ (Miller *et al.*, 1988). Although 8F-TMQ was 3.2 times more potent (EC_{50} = 0.44 μM) than TMQ (EC_{50} = 1.4 μM) as an antagonist of U46619-induced contractions on rat aortae, it possessed a 10-fold lower affinity

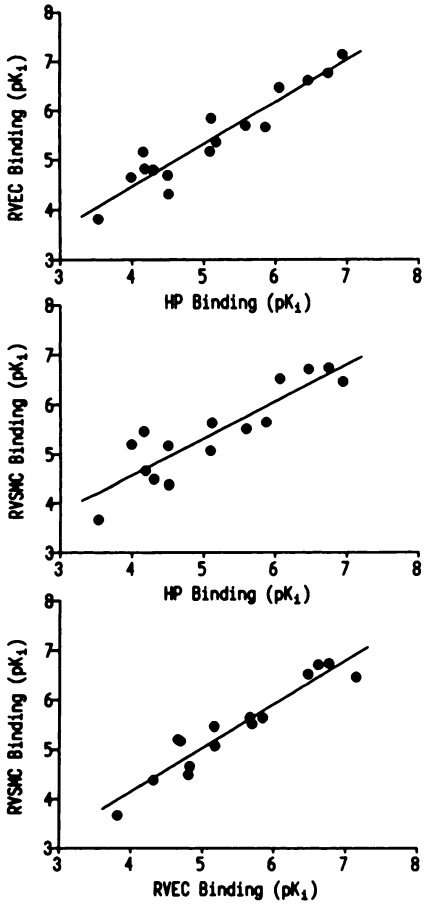


Fig. 3. Graphical correlation of receptor affinity (pK$_i$) of TMQ analogs for inhibition of specific [^{3}H]SQ 29548 binding to washed human platelets (HP) *vs.* cultured rat aortic vascular endothelial cells (RVEC) (upper panel); washed human platelets (HP) *vs.* cultured rat aortic vascular smooth muscle cells (RVSMC) (middle panel); and cultured rat aortic endothelia cells (RVEC) versus rat aortic vascular smooth muscle cells (RVSMC) (lower panel). Each point is the mean value of 3 to 31 experiments performed in three different preparations.

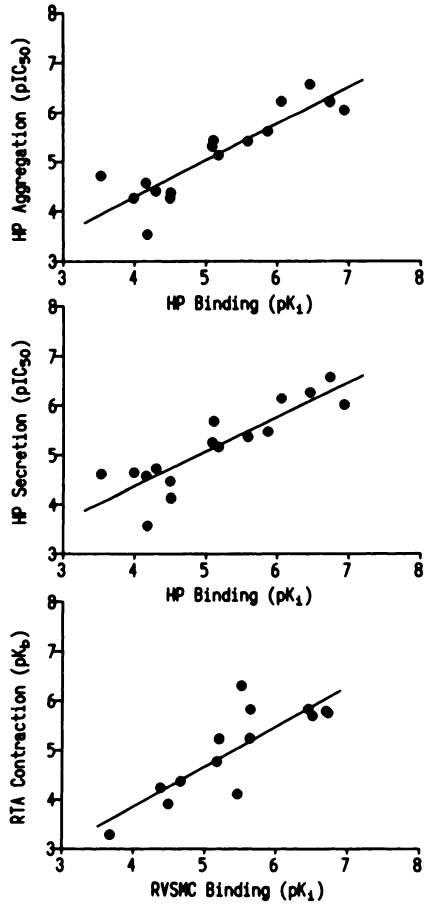


Fig. 4. Correlations of inhibitory potency values (pK$_i$ or pK$_b$) of TMQ analogs for inhibition of specific [^{3}H]SQ 29548 binding to washed human platelets (HP) *vs.* U46619-induced aggregation (upper panel) and serotonin secretion (middle panel) in human platelet-rich plasma, and inhibition of specific [^{3}H]SQ 29548 binding to cultured rat aortic vascular smooth muscle cells (RVSMC) *vs.* inhibition of U46619-induced vasoconstriction in rat thoracic aortae (RTA) (lower panel). pIC$_{50}$ or pK$_b$ values were taken from our previous papers. Each point is the mean value of at least four experiments and is analyzed by a linear regression analysis.

for TP receptors in smooth muscle cells (table 1). Furthermore, the optical isomers of 8F-TMQ showed a high stereoselectivity (R $\gg$ S) for binding to TP receptors on platelets (35-fold difference) and smooth muscle cells (11-fold difference), and they produced qualitatively different functional effects on rat aortae. Whereas R-8F-TMQ was 14 times more potent than the S-isomer as a TP antagonist of U46619-induced responses on platelets, only the S-isomer (EC_{50} = 1.9 μM) was an agonist in rat aorta, and the contractile responses were not blocked by SQ 29548 (< 1 μM) (Markovich *et al.*, 1992). Further, there was a 17-fold difference between receptor affinity and functional activity for the S-isomer of 8F-TMQ in smooth muscle cells and rat aorta. Previous reports also indicate that certain TP prostanoid antagonists exhibit contractile activities in vascular smooth muscle systems (Lefer *et al.*, 1980; Morinelli *et al.* 1989). Thus, the functional selectivities of these halogenated TMQ analogs are independent of TP receptor affinities, and may be associated, in part, with differences in tissue distribution or metabolism of the TMQ analogs or to an interaction of the TMQ analogs with other prostanoid receptor sites in vascular smooth muscle.

Nonprostanoid TMQ analogs did not discriminate between TP receptor populations in platelets and vascular cells. This finding agrees favorably with earlier radioligand binding studies of structurally diverse TP receptor agonists and antagonists in platelets and vascular tissues (Hanasaki *et al.*, 1988a,b; Mihara *et al.*, 1989). However, unlike TMQ analogs, structural derivatives of the 13-azapinane series of TXA_2 analogs differentiate between TP receptor populations present in platelets and vascular smooth muscle cells (Masuda *et al.*, 1991). The inability of TMQ analogs to differentiate between these subclasses of TP receptors may be related to the chemical nature of the radioligand used (Masuda *et al.*, 1991), or to an interaction with TP receptor domains that do not reveal heterogeneity between these proposed subclasses.

The recent cloning and expression of human TP receptors (Hirata *et al.*, 1991) also led to a proposal that a high degree of homology exists for TP receptor in platelets and vascular tissues. We have reported that the isomers of TMQ show a similar stereodependency (R-isomer $\gg$ S-isomer) for displacement[^{3}H]SQ 29548 from TP binding sites in platelets, vascular endothelial and smooth muscle cells of rat (Shin *et al.*, 1992), and further, that the rank orders of potencies for halogenated TMQ analogs are the same in rat versus human platelets (Romstedt *et al.*, 1992). In the analysis of structure-affinity

relationships for the TMQ analogs (fig. 3), the slope values of correlations in human platelets versus rat vascular endothelial cells, and in human platelets versus rat vascular smooth muscle cells, deviated from unity. Thus, small intertissue or species differences may exist in the binding of TMQ analogs to TP receptor populations in these cell types. Indeed, Ogletree and Allen (1992) reported that R(+)-TMQ does not inhibit the U46619-induced contraction of guinea pig aorta and portal vein, indicating that differences may exist between TMQ and other nonprostanoid or prostanoid TP receptor antagonists in these preparations. Until the receptors from different cell types are cloned and expressed, it is not possible to conclude whether TP receptors in these tissues are different. Nevertheless, these nonprostanoid TMQ analogs may be useful to resolve the underlying basis for the observed species and intertissue differences in TP receptor populations.

TP receptors have been identified in rat vascular endothelial and bovine pulmonary artery and bovine aortic endothelial cells (Hanasaki *et al.*, 1988a; Hunt *et al.*, 1992; Sung *et al.*, 1989), and Yamada *et al.* (1989) reported that platelet aggregation induced by TXA_2 was inhibited in the presence of human umbilical vein endothelial cells; this action may be related to prostacyclin synthesis or release from endothelial cells. In our studies, rat vascular endothelial cells, but not human umbilical endothelial venous cells, were found to contain a population of high affinity TP receptors. Because specific [^{3}H]SQ 29548 binding was not observed in human umbilical venous endothelial cells studied under the same experimental conditions as rat vascular endothelial cells, we suggest that human endothelial cells do not contain TP receptor binding sites. This interpretation should be viewed with caution, inasmuch as the isolated human umbilical venous endothelial cells may not express or lose TP receptors under the culturing conditions. Thus, the question of species or tissue differences in the distribution of TP receptors in endothelial cells remains to be elucidated.

Our results demonstrate that the nonprostanoid TMQ analogs inhibited specific [^{3}H]SQ 29548 binding in a concentration-dependent and highly stereoselective manner (R-isomer $\gg$ S-isomer) in human platelets and rat cultured vascular endothelial and smooth muscle cells. The stereodependency of the TMQ isomers (564- to 933-fold isomeric activity ratio; see table 1) is at least a log unit greater than that reported for isomers of 13-azaprostanoic acid (Huzoor-Akbar *et al.*, 1985), an indolepropanoic acid derivative (L-654, 664) (Mais *et al.*, 1990; Masuda *et al.*, 1991) or the related prostanoid analogs, S-145 (Dube *et al.*, 1989), difluorothromboxane (Morinelli *et al.*, 1989) and 13-azapinane analogs (PTA-OH and ONO 11120) (Masuda *et al.*, 1991). Thus, the optical isomers of TMQ should be valuable as nonprostanoid, stereospecific probes to characterize TP receptors.

A comparison of the rank orders of radioligand binding inhibition indicates that nonprostanoid TMQ analogs exhibit similar affinities for TP receptors in platelets, endothelial and smooth muscle cells. Our findings indicate that: 1) recognition sites of TMQ analogs reflect the pharmacological relevancy of TP receptors in both human platelets and rat vascular smooth muscle cells; 2) nonprostanoid TMQ analogs recognize the high affinity site that is labeled by a TP receptor antagonist, SQ 29548, with the same specificity in human platelets, rat vascular endothelial cells and vascular smooth muscle cells; 3) functional tissue selectivities of halogenated TMQ analogs in aorta may not be explained by differences in affinities for TP receptor populations; and 4) enantiomeric halogenated TMQ analogs that possess high receptor affinity for TP receptors should be useful as affinity labels for the characterization of receptor binding domains for this nonprostanoid chemical class of compounds in these cells.

Acknowledgment

This research is part of a Ph.D. dissertation (Yangmee Shin) submitted to the Graduate School of The Ohio State University (1991).

References

AHN, C. -H., ROMSTEDT, K. J., WALLACE, L. J., MILLER, D. D. AND FELLER, D. R.: Characterization of the inhibition of U46619-mediated human platelet activation by the trimetoquinol isomers: Evidence for endoperoxide/thromboxane A_2 receptor blockade. Biochem. Pharmacol. **37:** 3023–3033, 1988.

ARMSTRONG, R. A., JONES, R. L. AND WILSON, N. H.: Ligand binding to thromboxane receptors on human platelets: correlation with biological activity. Br. J. Pharmacol. **79:** 953–964, 1983.

CHENG, Y. AND PRUSOFF, W. H.: Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (I_{50}) of an enzymatic reaction. Biochem. Pharmacol. **22:** 3099–3108, 1973.

COLE, O. F., FAN, T. -P. AND LEWIS, G. P.: Isolation characterization, growth and culture of endothelial cells from the rat aorta. Cell Biol. Int. Rep. **10:** 399–405, 1986.

DORN, G. W., II: Distinct platelet thromboxane A_2/prostaglandin H_2 receptor subtypes. J. Clin. Invest. **84:** 1883–1891, 1989.

DORN, G. W., II: Tissue- and species-specific differences in ligand binding to thromboxane A_2 receptors. Am. J. Physiol. **261:** R145–R153, 1991.

DUBE, G. P., KURTZ, W., MAIS, D. E., BRUNE, K., FLEISCH, J. AND JAKUBOWSKI, J. A.: In vitro profile of a novel thromboxane A_2 antagonist in platelets and arteries. American Heart Meeting, 62nd Scientific Session, November 13–16, p. 259, 1989.

FEDYNA, J. S., ADEJARE, A., MILLER, D. D. AND FELLER, D. R.: Pharmacological evaluation of the β-adrenoceptor agonist and thromboxane antagonist properties of N-substituted trimetoquinol analogues. Eur. J. Pharmacol. **135:** 161–171, 1987.

FURCI, L., FITZGERALD, D. J. AND FITZGERALD, G. A.: Heterogeneity of prostaglandin H_2/thromboxane A_2 receptors: distinct subtypes mediate vascular smooth muscle contraction and platelet aggregation. J. Pharmacol. Exp. Ther. **258:** 74–81, 1991.

HALUSHKA, P. V. AND LEFER, A. M.: Thromboxane A_2 in health and disease. Fed. Proc. **46:** 131–158, 1987.

HANASAKI, K., NAKANO, K., KASAI, H., KURIHARA, H. AND ARITA, H.: Identification of thromboxane A_2 receptor in cultured vascular endothelial cells of rat aorta. Biochem. Biophys. Res. Commun. **151:** 1352–1357, 1988a.

HANASAKI, K., NAKANO, K., KASAI, H., ARITA, H., OHTANI, K. AND DOTEUCHI, M.: Specific receptors for thromboxane A_2 in cultured vascular smooth muscle cells of rat aorta. Biochem. Biophys. Res. Commun. **150:** 1170–1175, 1988b.

HANASAKI, K., NAKANO, K., KASAI, H. AND ARITA, H.: Biochemical characterization and comparison of rat thromboxane A_2/prostaglandin H_2 receptors in platelets and cultured aortic smooth muscle cells. Biochem. Pharmacol. **38:** 1967–1976, 1989.

HANASAKI, K. AND ARITA, H.: Characterization of thromboxane A_2/prostaglandin H_2 receptor (TXA_2/PGH_2) receptors of rat platelets and their interaction with TXA_2/PGH_2 receptor antagonists. Biochem. Pharmacol. **37:** 3923–3929, 1988.

HANASAKI, K. AND ARITA, H.: A common binding site for primary prostanoids in vascular smooth muscle: A definitive discrimination of the binding for thromboxane A_2/prostaglandin H_2 receptor agonist from its antagonist. Biochim. Biophys. Acta. **1013:** 28–35, 1989.

HARROLD, M. W., GERHARDT, M. A., ROMSTEDT, K. J., FELLER, D. R. AND MILLER, D. D.: Synthesis and platelet antiaggregatory activity of trimetoquinol analogs as endoperoxide/thromboxane A_2 antagonists. Drug. Des. Deliv. **1:** 193–207, 1987.

HARROLD, M. W., GRAJZL, B., SHIN, Y., ROMSTEDT, K. J., FELLER, D. R. AND MILLER, D. D.: Synthesis and thromboxane A_2 antagonist activity of N-benzyltrimetoquinol analogues. J. Med. Chem. **31:** 1506–1512, 1988.

HEDBERG, A., HALL, S., OGLETREE, M. L., HARRIS, D. N. AND LIU, E. C. K.: Characterization of [5,6-^{3}H] SQ 29548 as a high affinity radioligand binding to thromboxane A_2/prostaglandin H_2 receptors in human platelets. J. Pharmacol. Exp. Ther. **245:** 786–792, 1988.

HIRATA, M., HAYASHI, Y., USHIKUBI, F., YOKOTA, Y., KAGEYAMA, R., NAKANISHI, S. AND NARUMIYA, S.: Cloning and expression of cDNA for human thromboxane A_2 receptor. Nature **349:** 617–620, 1991.

HUNT, J. A., MERRITT, J. E., MACDERMOT, J. AND KEEN, M.: Characterization of the thromboxane receptor mediating prostacyclin release from cultured endothelial cells. Biochem. Pharmacol. **43:** 1747–1752, 1992.

HUZOOR-AKBAR, MUKHOPADHYAY, A., ANDERSON, K. S., NAVRAN, S. S., ROMSTEDT, K., MILLER, D. D. AND FELLER, D. R.: Antagonism of prostaglandin-mediated responses in platelets and vascular smooth muscle by 13-azaprostanoic acid analogs. Biochem. Pharmacol. **34:** 641–647, 1985.

KATTLEMAN, E. J., VENTON, D. L. AND LEBRETON, G. C.: Characterization of U46619 binding in unactivated intact human platelets and determination of

binding site affinities of four TXA_2/PGH_2 receptor antagonists. Thromb. Res. **41:** 471–481, 1986.

LEDUC, L., WYCHE, A., SPRECHER, H., SANKARAPPE, S. AND NEEDLEMAN, P.: Analogues of arachidonic acid used to evaluate structural determinants of prostaglandin receptor and enzyme specificities. Mol. Pharmacol. **19:** 242–247, 1981.

LEFER, A., SMITH, E., ARAKI, H., SMITH, J., AHARONY, D., CLAREMAN, D., MAGOLDA, R. AND NICOLAOU, K.: Dissociation of vasoconstrictor and platelet aggregatory activities of thromboxane by carbacyclic thromboxane A_2. Proc. Acad. Natl. Sci. USA **77:** 1706–1710, 1980.

LEFER, A. M.: Comparison of the actions of thromboxane receptor antagonists in biological systems. Drugs Today **21:** 283–291, 1985.

MAIS, D. E., DEHALL, D., SIGHTLER, H., HALUSHKA, P. V.: Different pharmacologic activities for 13-azapinane thromboxane A_2 analogs in platelets and blood vessels. Eur. J. Pharmacol. **148:** 309–315, 1988.

MAIS, D. E., DUNLAP, C., HAMANAKA, N. AND HALUSHKA, P. V.: Further studies on the effects of epimers of thromboxane A_2 antagonists on platelets and veins. Eur. J. Pharmacol. **111:** 125–128, 1985a.

MAIS, D. E., SAUSSY, D. L., CHAIKHOUVI, A., KOCHEL, P. J., KNAPP, D. R., HAMANAKA, N. AND HALUSHKA, P. V.: Pharmacologic characterization of human and canine thromboxane A_2/prostaglandin H_2 receptors in platelets and blood vessels: evidence for different receptors. J. Pharmacol. Exp. Ther. **233:** 418–424, 1985b.

MAIS, D. E., LIEL, N. AND HALUSHKA, P. V.: Photoaffinity receptor antagonist for human platelet thromboxane A_2/prostaglandin H_2 receptors. Biochem. Pharmacol. **40:** 1457–1461, 1990.

MARKOVICH, K. M., V. TANTISHAIYAKUL, V., HAMADA, A., MILLER, D. D., ROMSTEDT, K. J., SHAMS, G., SHIN, Y., FRAUNDORFER, P. F., DOYLE, K. AND FELLER, D. R.: Synthesis of halogenated trimetoquinol derivatives and evaluation of the β-agonist and thromboxane A_2 antagonist activities. J. Med. Chem. **35:** 466–479, 1992.

MASUDA, A., MAIS, D. E., OATIS, JR., J. E. AND HALUSHKA, P. V.: Platelet and vascular thromboxane A_2/prostaglandin H_2 Receptors: Evidence for different subclasses in the rat. Biochem. Pharmacol. **42:** 537–544, 1991.

MAYO, J. R., NAVRAN, S. S., HUZOOR-AKBAR, MILLER, D. D. AND FELLER, D. R.: Stereodependent inhibition of human platelet function by the optical isomers of trimetoquinol. Biochem. Pharmacol. **30:** 2237–2241, 1981.

MCGUIRE, P. G. AND ORKIN, R. W.: Isolation of rat aortic endothelial cells by primary explant techniques and their phenotypic modulation by defined substrata. Lab. Invest. **57:** 94–105, 1987.

MCPHERSON, G.: A collection of radioligand analysis program. *In* Manual of Kinetic, EBDA, Ligand, Lowry, Elsevier-BIOSOFT, Cambridge, UK, 1985.

MIHARA, S., HARA, S., UEDA, M., IDE, M. AND FUJIMOTO, M.: Comparison of vascular and platelet thromboxane A_2/prostaglandin H_2 receptors in the pig. Eur. J. Pharmacol. **160:** 313–321, 1989.

MILLER, D. D., CLARK, M. T., ADEJARE, A., NEIDERT, K., HAMADA, A., SHAMS, G., ROMSTEDT, K. J. AND FELLER, D. R.: Fluorotetrahydroisoquinolines as adrenergic and antithrombotic agents. *In* Progress in Catecholamine Research (Part A), Alan R. Liss, Inc., pp. 403–407, 1988.

MORINELLI, T. A., OKWU, A. K., MAIS, D. E., HALUSHKA, P. V., JOHN, V., CHEN, C. -K. AND FRIED, J.: Difluorothromboxane A_2 and stereoisomers: Stable derivatives of thromboxane A_2 with differential effects on platelets and blood vessels. Proc. Natl. Acad. Sci. USA **86:** 5600–5604, 1989.

MORINELLI, T. A., MAIS, D. E., OATIS, J. E., CRUMBLEY, A. J. AND HALUSHKA, P. V.: Characterization of thromboxane A_2/prostaglandin H_2 receptors in human vascular smooth muscle cells. Life Sci. **46:** 1765–1772, 1990.

MUKHOPADHYAY, A., NAVRAN, S. S., AMIN, H. M., ABDEL-AZIZ, S. A., CHANG, J., SOBER, D. J., MILLER, D. D. AND FELLER, D. R.: Effect of trimetoquinol analogs for antagonism of endoperoxide thromboxane A_2-mediated responses in human platelets and rat aorta. J. Pharmacol. Exp. Ther. **232:** 1–9, 1985.

OGLETREE, M. L. AND ALLEN, G. T.: Interspecies differences in thromboxane receptors: studies with thromboxane receptor antagonists in rat and guinea-pig smooth muscles. J. Pharmacol. Exp. Ther. **260:** 789–794, 1992.

POLLAK, O. AND KASAI, T.: Appearance and behavior of aortic cells in vitro. Am. J. Med. Sci. **248:** 71–78, 1964.

ROMSTEDT, K. J., SHIN, Y., MILLER D. D. AND FELLER, D. R.: Comparison of thromboxane A_2 (TX) receptors in human and rat platelets using [^{3}H]SQ,548 (HSQ) and trimetoquinol analogs (Abstract). The Pharmacologist **34:** 197, 1992.

ROSS, R.: The smooth muscle cell. II. Growth of smooth muscle in culture and formation of elastic fibers. J. Cell Biol. **50:** 172–186, 1971.

SHAMS, G., ROMSTEDT, K. J., GERHARDT, M. A., HARROLD, M. W., MILLER, D. D. AND FELLER, D. R.: Pharmacological evaluation of the β-adrenoceptor agonist and thromboxane receptor blocking properties of 1-benzyl substituted trimetoquinol analogues. Eur. J. Pharmacol. **184:** 21–31, 1990.

SHIN, Y., ROMSTEDT, K. J., DOYLE, K., HARROLD, M. W., GERHARDT, M. A., MILLER, D. D., PATIL, P. N. AND FELLER, D. R.: Pharmacologic antagonism of thromboxane A_2 receptors by trimetoquinol analogs in vitro and in vivo. Chirality **3:** 112–117, 1991.

SHIN, Y., ROMSTEDT, K. J., MARKOVICH, K. M., MILLER, D. D. AND FELLER D. R.: Stereospecific interactions of nonprostanoid trimetoquinol analogs with thromboxane A_2/prostaglandin H_2 receptor sites in human and rat platelets, and rat vascular endothelial and smooth muscle cells. Pharmacology Communications **1:** 303–312, 1992.

SUNG, C. -P., ARLETH, A. J. AND BERKOWITZ, B. A.: Endothelial thromboxane receptors: Biochemical characterization and functional implications. Biochem. Biophys. Res. Commun. **158:** 326–333, 1989.

SWAYNE, G. T. G., MAGUIRE, J., DOLAN, J., RAVAL, P., DANE, G., GREENER, M. AND OWEN, D. A. A.: Evidence for homogeneity of thromboxane A_2 receptor using structurally different antagonists. Eur. J. Pharmacol. **152:** 311–319, 1988.

TAKAHARA, K., MURRAY, R., FITZGERALD, G. A. AND FITZGERALD, D. J.: The response to thromboxane A_2 analogues in human platelets. J. Biol. Chem. **265:** 6836–6844, 1990.

TYMKEWYCZ, P. M., JONES, R. L., WILSON, N. H. AND MARR, C. G.: Heterogeneity of thromboxane A_2 (TP) receptors: evidence from antagonist but not agonist potency measurements. Br. J. Pharmacol. **102:** 607–614, 1991.

YAMADA, Y., FURUI, H., FURUMICHI, T., SUZUKI, T., YAMAUCHI, K., MITSUHIRO, Y., HAYASHI, H. AND SAITO, H.: Inhibitory effects of endothelial cells and calcium channel blockers on platelet aggregation. Jpn. Heart J. **31:** 201–215, 1990.

Send reprint requests to: Dennis R. Feller, Ph.D., Division of Pharmacology, College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210-1291.