
UNIVERSITE DE LAUSANNE - FACULTE DE BIOLOGIE ET DE MEDECINE

Département de Médecine Interne

Division de Physiopathologie Clinique

**No implication of thromboxane-prostanoid receptors
in reactive hyperemia of skin and skeletal muscle in
human forearm**

THESE

préparée sous la direction du Professeur Bernard WAEBER

et présentée à la Faculté de biologie et de médecine de
l'Université de Lausanne pour l'obtention du grade de

DOCTEUR EN MEDECINE

par

Antoine PASCHE

Médecin diplômé de la Confédération Suisse
Originaire de Oron-la-Ville (Vaud)

Lausanne

2013

1

Bibliothèque Universitaire
de Médecine / BiUM
CHUV-BH08 - Bugnon 46
CH-1011 Lausanne

WG
106
PAS

BKTE 3690

0 4470 3 268

Imprimatur

Vu le rapport présenté par le jury d'examen, composé de

Directeur de thèse Monsieur le Professeur Bernard Waeber

Co-Directeur de thèse

Expert Monsieur le Professeur Daniel Hohl

*Directrice de l'Ecole Madame le Professeur Stephanie Clarke
doctorale*

la Commission MD de l'Ecole doctorale autorise l'impression de la thèse de

Monsieur Antoine Pasche

intitulée

*No implication of thromboxane-prostanoid receptors in
reactive hyperemia of skin and skeletal muscle in human
forearm*

Lausanne, le 15 janvier 2013

*pour Le Doyen
de la Faculté de Biologie et de Médecine*



*Madame le Professeur Stephanie Clarke
Directrice de l'Ecole doctorale*

REMERCIEMENTS

Je tiens à remercier tout particulièrement le Professeur François Feihl, qui par sa disponibilité et sa gentillesse, a su faire progresser et aboutir ce travail.

Je remercie également le Professeur Bernard Waeber pour son soutien, Madame Françoise Bilat pour son humour et toute l'équipe du laboratoire de physiopathologie clinique pour tous les moments passés au 19ème étage du CHUV.

RESUME

L'hyperhémie réactive, définie comme l'augmentation transitoire du flux sanguin après une courte période d'ischémie, pourrait être influencée par des vasoconstricteurs de la famille des prostanoïdes, telle que la thromboxane.

Le terutroban (S18886) est un antagoniste spécifique des récepteurs à la thromboxane. L'étude présentée a cherché à déterminer l'effet du terutroban sur l'hyperhémie réactive dans la peau et le muscle squelettique de l'avant-bras de volontaires sains.

Vingt volontaires sains ont été randomisés en aveugle pour recevoir oralement 30mg/j de terutroban ou un placebo pendant 5 jours puis réciproquement pendant une deuxième période de 5 jours, selon un schéma *cross-over*. L'ischémie transitoire a été provoquée par l'occlusion de l'artère brachiale par une manchette gonflée au dessus de la pression systolique. L'hyperhémie réactive était évaluée dans les tissus de l'avant-bras, en mesurant le flux sanguin, pour la peau par une méthode laser Doppler, et pour le muscle au moyen d'une pléthysmographie par jauge de contrainte durant une occlusion veineuse.

Au premier et au dernier jour de chaque période de traitement, l'hyperhémie réactive était mesurée avant et 2 heures après l'ingestion du comprimé.

Que ce soit dans la peau ou le muscle, le terutroban n'a pas montré d'effet sur le flux de pic post-occlusion ni sur la réponse globale d'hyperhémie, exprimée en aire sous la courbe.

En conclusion, dans la peau et le muscle de sujets sains, l'hyperhémie réactive n'est pas influencée par les récepteurs spécifiques à la thromboxane.

No Implication of Thromboxane Prostanoid Receptors in Reactive Hyperemia of Skin and Skeletal Muscle in Human Forearm

Antoine Pasche, MD,* Abigail Heim, MD,* Lucas Liaudet, MD,† Bernard Waeber, MD,* and François Feihl, MD*

Abstract: There is evidence that reactive hyperemia (ie, the transient increase of blood flow above resting level after a short period of ischemia) could be negatively modulated by vasoconstrictor prostanoids. The present study tested whether pharmacological blockade of the thromboxane prostanoid receptors with the specific antagonist S18886 (terutroban) would amplify reactive hyperemia in human skin and skeletal muscle. Twenty healthy young male volunteers were enrolled in a randomized, blinded, crossover trial of oral S18886 30 mg/d for 5 days versus placebo. Reactive hyperemia was evaluated in forearm skin and skeletal muscle, after occlusion of the brachial artery with a pneumatic cuff inflated at suprasystolic pressure. Blood flow was measured with laser Doppler imaging (skin) and strain gauge venous occlusion plethysmography (muscle). On the first and last day of each treatment period, recordings of reactive hyperemia were obtained immediately before and 2 hours after drug intake. Whether in forearm muscle or skin, S18886 had no discernible effect on peak postocclusion blood flow, nor on the global hyperemic response as quantified by the area under curve. These results do not support that thromboxane prostanoid receptor activation could exert a moderating influence on reactive hyperemia in human skin and skeletal muscle, at least in young subjects.

Key Words: cyclooxygenase, thromboxane A₂, reactive hyperemia

(*J Cardiovasc Pharmacol*TM 2013;61:127–132)

INTRODUCTION

Reactive hyperemia (or synonymously *postischemic vasodilation*) refers to the transient vasodilation taking place in many tissues when perfusion is reestablished after a short interruption. In man, reactive hyperemia is relatively simple to evaluate in forearm muscle and skin, where reliable non-invasive methods exist for the measurement of blood flow (respectively, strain-gauge plethysmography and laser Doppler flowmetry), whereas transient ischemia can be obtained easily by the inflation at suprasystolic pressure of a pneumatic

cuff placed on the upper arm.^{1,2} Several studies have indicated that, recorded in this way, reactive hyperemia is linked to and thus could be used as a surrogate marker for cardiovascular risk.^{3–11} However, one obstacle to such use might be the fact that many subjects at increased cardiovascular risk receive cyclooxygenase (COX) inhibitors, with a potential confounding influence on the measured vascular responses.

COX catalyzes the transformation of arachidonic acid to prostaglandin H₂, a common initial step that then leads, through multiple downstream pathways, to the formation of several biologically active compounds collectively termed prostanoids. There are essentially 2 isoforms of COX, COX-1, and COX-2, each coded by a different gene. Both can be found in a wide array of tissues, including the vascular endothelium and vascular smooth muscle.¹² Classically, the expression of COX-1 is constitutional, whereas COX-2 is induced by inflammation, but the presence of COX-2 has also been described in the absence of inflammatory stimulus, notably in endothelial cells.¹² In humans, the development of hypertension seems associated with a shift in prostanoid production from predominant control by COX-1 to predominant control by COX-2.¹² Prostanoids exert their biological effects by stimulating at least 5 different types of receptors, the IP, DP, EP, FP, and thromboxane prostanoid (TP).¹³ Classically, the prostanoid PGI₂ (prostacyclin) specifically ligates the IP receptor, leading in blood vessels to vasodilation and inhibition of coagulation, whereas thromboxane A₂ (TxA₂) stimulates the TP receptors, with the opposite consequences. The increased cardiovascular risk associated with the intake of some pharmacological COX inhibitors, such as rofecoxib (selective for COX-2) or diclofenac (nonselective) has been explained by an induced shift in the balance of PGI₂ and TxA₂ in favor of the latter.^{14,15} The physiology and pathophysiology of prostanoids is rendered more complex by the fact that some of these compounds may ligate more than 1 receptor type, potentially leading to conflicting effects. A case in point is that of prostaglandin D₂, which may stimulate both endothelial DP receptors (leading to endothelium-dependent vasodilation) and vascular smooth muscle TP receptors (endothelium-independent vasoconstriction).¹²

In view of this complexity, it is not surprising that, in healthy humans, the impact of COX inhibitors on the reactive hyperemia of skin^{9,16–21} or forearm muscle^{21–25} has been variable, ranging from marked or modest blunting,^{21–25} no effect,^{18,19} to marked potentiation.²⁰ One potential reason for these discrepancies might reside in differences between studies regarding the

Received for publication August 20, 2012; accepted October 16, 2012.

From the *Division de Physiopathologie clinique; and †Service de Médecine intensive adulte, Centre Hospitalier Universitaire Vaudois and Université de Lausanne, Lausanne, Switzerland.

Supported and monitored by Institut de Recherches internationales Servier (the manufacturer of S18886), Courbevoie, France.

The authors report no conflicts of interest.

Reprints: Prof François Feihl, MD, PPA, BH10-701, CHUV, CH-1011 Lausanne, Switzerland (e-mail: bernard.waeber@chuv.ch).

Copyright © 2013 by Lippincott Williams & Wilkins

relative impact of the administered drug on the production of vasoconstrictive versus vasodilator COX products. To examine this possibility, it would be necessary to selectively block the action of vasoconstrictor prostanoids.

In human forearm, a short transient ischemia (3–10 minutes) is followed by an increased concentration in effluent venous blood of thromboxane B₂, the stable metabolite of TxA₂.^{26–29} Thus, in this vascular territory at least, the stimulation of TP receptors might contribute to limit reactive hyperemia. In the present study, we hypothesized that administration to healthy volunteers of S18886 (terutroban), a selective antagonist of TP receptors,^{30,31} would enhance reactive hyperemia in forearm skin and muscle.

MATERIALS AND METHODS

Subjects

All participants were fully informed about the protocol of the study and gave their written consent. The study conformed to the principles outlined in the Declaration of Helsinki and was approved by the institutional review board and by the Swiss regulatory authorities (Swissmedic).

The study took place from April 5, 2007, to November 21, 2007. Twenty white male healthy subjects, aged 18–30 years, were enrolled. Inclusion criteria were an inconspicuous medical history without bleeding disorder, no remarkable findings on clinical examination, and normal results on the following laboratory tests: blood cell count, potassium, sodium, calcium, creatinine, total protein, glucose, liver, pancreatic, and coagulation (prothrombin time, partial prothromboplastin time, fibrinogen, and platelet function), platelet function assessed with the PFA-100 System (Siemens Healthcare, Erlangen, Germany). Additional requirements were negative serology for hepatitis B and C virus and HIV, normal electrocardiogram, negative urine analysis for blood (disptest), and drugs (minimal test).

Exclusion criteria were a planned strenuous activity during the study period, surgery in the 3 months before the study or planned during the study, excessive use of alcohol (>45 g/d), excessive intake of caffeine beverage (≥5 cups/d or ≥500 mg of caffeine/d), use of any addictive substance, regular use of agents suppressing gastric acidity, and use any drugs affecting hemostasis in the 10 days preceding inclusion (anticoagulant agents, antiplatelet drugs, steroidal and nonsteroidal anti-inflammatory drugs, and drug containing salicylates).

Assessment of Reactive Hyperemia

Reactive hyperemia was elicited in the forearm by temporarily occluding the ipsilateral brachial artery with a standard size pneumatic cuff placed around the upper arm and inflated above systolic pressure (200 mm Hg). For skin assessment, occlusion times of 3 minutes and then 30 seconds were used, with 5 minutes of reperfusion allowed in between. For muscle assessment, the occlusion time was 5 minutes. Separate maneuvers were carried out on the dominant side and the nondominant side for the recording of reactive hyperemia in forearm muscle and forearm skin, respectively.

Forearm muscle blood flow was assessed using venous occlusion strain-gauge plethysmography (Hokanson, Bellevue, WA), with the hand excluded by means of a pneumatic cuff

inflated around the wrist, as described previously.²¹ Skin blood flow was measured on the volar face of the forearm, using a laser Doppler imager (LDI; Moor Instruments, Axminster, United Kingdom), as described previously.^{2,21}

Study Design and Protocol

The study was single center, placebo controlled, crossover, and triple blinded (participants, investigators, and data collector). For each participant, the sequence of treatment (active drug first or placebo first) was randomly assigned at inclusion. To ensure that the treatment was given blindly after randomization, tablets of identical appearance containing either placebo or the active drug were prepared by the Institut de Recherches internationales Servier (Courbevoie, France) and sent to our center. The active drug was the TxA₂ receptor blocker S18886 (terutroban), 30 milligrams per tablet.

The complete protocol comprised 1 selection and 4 investigation sessions. The various eligibility and exclusion criteria were checked on the selection session and rechecked 7 days later on investigation session 1 (Fig. 1A). Randomization took place on investigation session 1. The first treatment period lasted from investigation session 1 to investigation session 2, 5 days later. A washout period of 10–15 days followed. The second treatment period then started on investigation session 3 and lasted 5 additional days, until investigation session 4. A single tablet containing S18886 30 mg or placebo was administered on each day of each treatment period, at 8:00 AM.

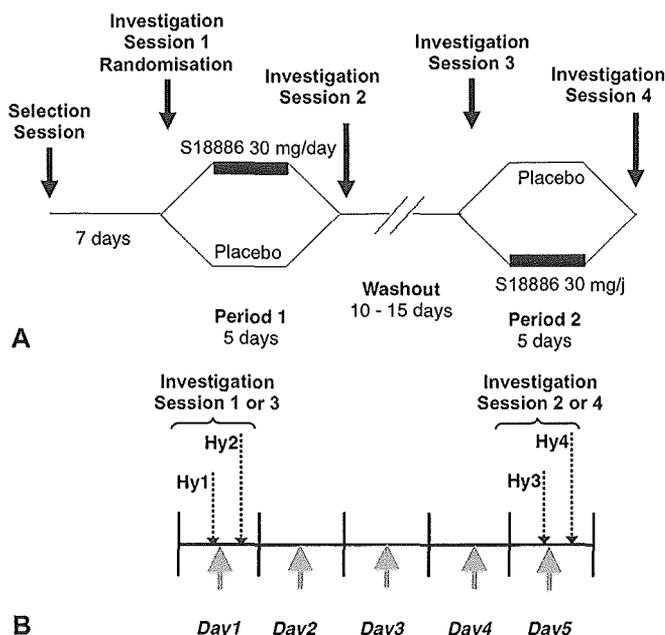


FIGURE 1. Study design. A, Crossover design, see text for explanations. B, Details of 5-day study period. Thick grey arrows designate oral intake of a single tablet containing either the active drug (S18886) or placebo. Stippled arrows labeled Hy1 to Hy4 designate recordings of reactive hyperemia in forearm muscle and skin. Hy1 carried out immediately before and Hy2 2 hours after intake of first tablet. Hy3 and Hy4 similarly bracket intake of the fifth tablet.

TABLE 1. Characteristics of Study Subjects

No. Subjects	20
Age, yrs	24.4 ± 2.5
Weight, kg	75.4 ± 8.1
Height, cm	181 ± 5
BMI, kg/m ²	23 ± 2.3
Body temperature, °C	36.5 ± 0.3
Heart rate, beats/min	63 ± 12
Blood pressure, mm Hg	
Systolic	124 ± 11
Diastolic	68 ± 13

Data are mean and SD.
BMI, body mass index.

To guarantee treatment compliance, the subjects had to report daily to the laboratory in the early morning, so that one investigator could hand over the appropriate tablet and verify its actual ingestion.

Starting from the selection session and for the entire duration of study, the subjects were instructed to avoid all the following: use any drugs affecting hemostasis (anticoagulant agents, antiplatelet drugs, steroidal and nonsteroidal anti-inflammatory drugs, and drug containing salicylates), use of any addictive substance, and use of agents suppressing gastric acidity. An additional requirement was to avoid any intake of caffeine beverage in the 24 hours preceding each session.

On each investigation session, reactive hyperemia in skin and muscle was examined immediately before and 2 hours after drug intake. In Figure 1B, these examinations are designated as Hy1 (beginning of treatment period, immediately before intake of the first tablet), Hy2 (2 hours after intake of first tablet), Hy3 (fifth day of treatment period, immediately before intake of the fifth tablet), and Hy4 (2 hours after intake of fifth tablet). The timing of Hy2 relative to Hy1 and Hy4 relative to Hy3 was based on the known pharmacodynamics of S18886. A single oral 30 mg dose of this drug affects maximal inhibition of platelet aggregation, starting after 30 minutes and maintained for at least 12 hours.^{30,31}

All investigations took place in a quiet room with air conditioning. Ambient temperature was kept between 21°C and 23.5°C. The subjects were examined in the supine position with arms supported by a cushion. On each vascular investigation, each type of reactive hyperemia (forearm muscle and forearm skin) was determined in triplicate, allowing 10 minutes between deflation of the arm cuff used for arterial occlusion and its next inflation. Determinations in forearm muscle were carried out first, on the dominant arm, and were followed by those in forearm skin, on the contralateral side.

Data Analysis

The recorded hyperemic response was summarized as (1) the baseline blood flow (ie, immediately before arterial occlusion), (2) the peak blood flow observed after release of arterial occlusion and (3) the area under curve, as previously described.²¹ Statistical analysis was carried out with mixed model analysis of variance, using treatment (placebo or

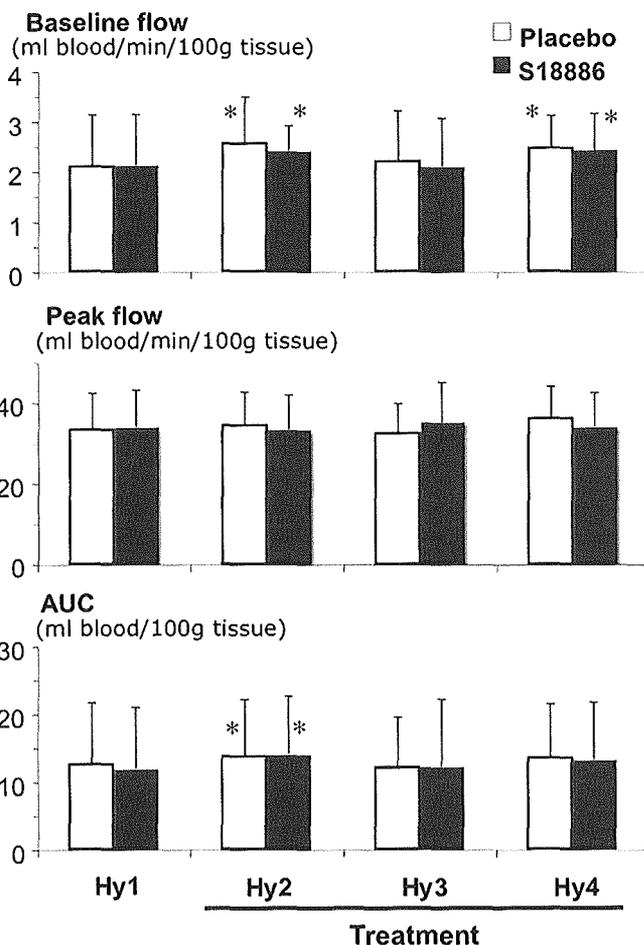


FIGURE 2. Reactive hyperemia in forearm muscle. Forearm blood flow measured with hand excluded, using venous occlusion strain gauge plethysmography. Blood flow interrupted for 5 minutes by pressurization at 200 mm Hg of a pneumatic cuff placed around the upper arm. Baseline flow measured immediately before cuff inflation. Peak flow maximal flow immediately after cuff deflation. AUC area under curve. Each subject went through two 5-day treatment periods. Treatment consisted in the daily ingestion of a single tablet containing 30 mg S18886 in one period and placebo in the other. Timing of measurements: Hy1 and Hy2 immediately before and 2 hours after intake of first tablet on first treatment day. Hy3 and Hy4 immediately before and 2 hours after intake of fifth tablet on fifth treatment day. * $p < 0.05$, ** $p < 0.01$ Hy2-Hy4 vs Hy1. None of the differences between treatments reached statistical significance. Data a means and SD, n = 20 subjects.

S18886), examination number (Hy1-Hy4), and their interactions as fixed factors repeated within subjects and subjects as random factors. The alpha level of all tests was set at 0.05. Data are summarized as mean and SD. All calculations were carried out with version 5 of the JMP software (SAS Institute, Cary, NC).

RESULTS

The demographic and hemodynamic characteristics of the remaining 20 subjects are shown in Table 1. Variables derived from reactive hyperemia in forearm muscle are

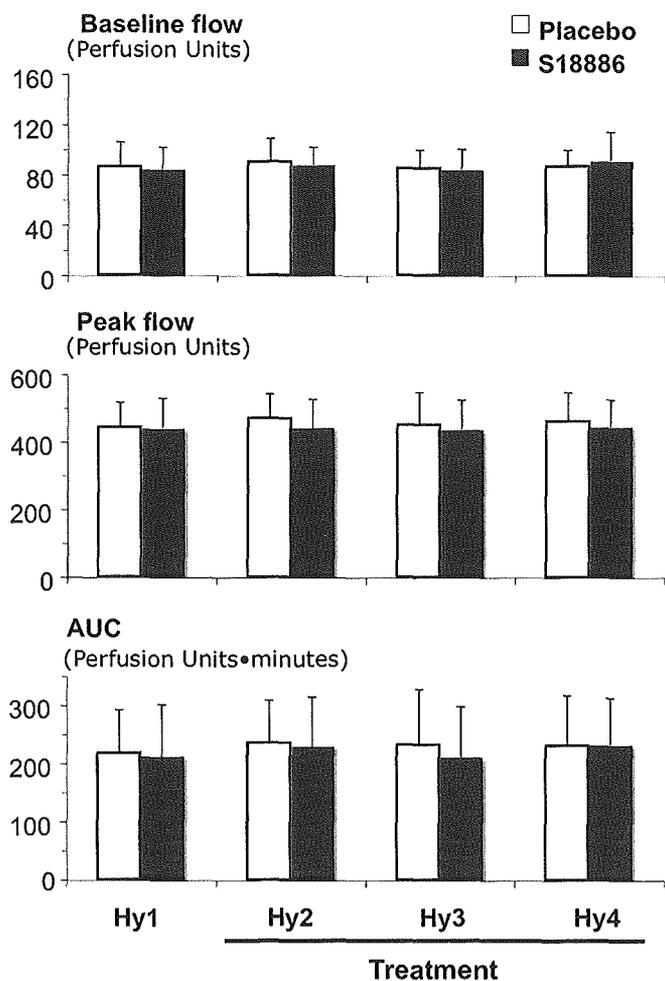


FIGURE 3. Reactive hyperemia in forearm skin. Skin blood flow measured on volar face of forearm. Blood flow interrupted for 3 minutes by pressurization at 200 mm Hg of a pneumatic cuff placed around the upper arm. See also legend for Figure 2. None of the differences between treatments reached statistical significance.

displayed in Figure 2. Identical results were obtained on the first examination of each treatment sequence (Hy1), irrespective of whether placebo or active drug was subsequently received. In both treatment sequences, a slight but statistically significant increase in baseline flow ($P = 0.016$) and area under curve ($P = 0.02$), but not peak flow, was noted 2 hours after ingestion of the first tablet (Hy2 vs. Hy1). A similar albeit not statistically significant trend occurred on the last day of each treatment sequence (ie, Hy4 vs. Hy2). The difference in baseline flow between Hy4 and Hy1 was statistically significant ($P = 0.029$). Most importantly, when compared with placebo, active treatment had no discernible effect on forearm muscle reactive hyperemia, at any time point (Hy2, Hy3, and Hy4).

The results regarding reactive hyperemia in forearm skin were essentially superimposable with those in forearm muscle, except for the absence of time-dependent effects. Again, there was no detectable influence of S18886, whether reactive hyperemia followed a 3-minute occlusion (Fig. 3) or 30-second occlusion (data not shown).

DISCUSSION

We present here the first study assessing the impact of TP receptor blockade on reactive hyperemia. Our results do not support any major modulation by TP receptor activation of the reactive hyperemia triggered by short periods of ischemia in the forearm skin and skeletal muscle microcirculation of young healthy humans.

In humans, several authors have explored the potential mediation of reactive hyperemia by vasoactive prostanoids, using the same investigation methods as in the present work and inhibition of COX with nonsteroidal anti-inflammatory agents (AINS) as pharmacological tools. As mentioned in the Introduction, and displayed more accurately in Table 2, results have been variable and at time inconsistent. When present at all, the observed effects of AINS administered systemically were toward a reduced amplitude and/or duration of postischemic vasodilation in muscle and skin. In contrast with these observations stands the study by Medow et al,²⁰ who used microdialysis to locally expose the calf skin of healthy volunteers to the AINS ketorolac and as a result observed a 2-fold amplification of reactive hyperemia. Because of the large concentration of ketorolac perfused into the microdialysis catheter (10 mM), these authors probably achieved higher tissue drug levels and thus more complete COX inhibition than was possible with systemic administration. The heterogeneity of results shown in Table 1 might thus be explained by differences between studies in the degree of COX inhibition, leading to different impacts on the balance of vasodilator and vasoconstrictor prostanoids. If this hypothesis is correct, then the data by Medow et al²⁰ would implicate vasoconstrictor prostanoids as major moderators of reactive hyperemia, at least in the skin.

TxA₂ is a major vasoconstrictor product of the COX pathway and, as reviewed in the Introduction, its production in human forearm appears stimulated by periods of ischemia as short (3–10 minutes) as carried out in the present study (skin 3 minutes, muscle 5 minutes). In these vascular beds, therefore, we anticipated that antagonizing the binding of TxA₂ to its specific TP receptor with S18886 would enhance postischemic vasodilation. This hypothesis was however not confirmed by our findings, which did not show any observable effect of S18886 on reactive hyperemia in the forearm (Figs. 2, 3), suggesting a lack of influence of TxA₂ on this physiological process.

One might argue that such a negative outcome might be related to an insufficient power of our study, due to the small size of the study population. However, a post hoc analysis carried out to verify this point indicated that, with the present design and 20 subjects included, power was ≥ 0.8 to detect a $\geq 10\%$ effect of treatment on either skin or muscle reactive hyperemia, had it been present on at least 1 of the 3 postdrug examinations. Thus, if our study missed an impact of S18886, as administered, on postischemic vasodilation, such impact is likely to be only minor.

A second issue to be considered is that of dosage and bioavailability of the study drug. Drug intake took place in the systematic presence of the investigator, so that treatment compliance was not in doubt. S18886 was administered orally as a single dose of 30 milligrams per day for 5 days. Measurements at Hy2 and Hy4 took place 2 hours after drug intake, a timing chosen because it corresponds to the time to

TABLE 2. Human Studies Concerning the Impact of COX Inhibitors on Reactive Hyperemia

Reference	Reactive Hyperemia		Inhibition of COX			Observed Effects on Reactive Hyperemia	
	Tissue	Ischemic Time, min	Agent	Route of Administration	Dosage	Peak Blood Flow	AUC
Kilbom and Wennmalm ²²	Muscle	5	Indomethacin	Rectal	1.25 mg/kg	25% decrease	50% decrease
Carlsson et al ²⁴	Muscle	3 and 5 1, 10, and 20	Ibuprofen	Oral	1200 mg	20%–25% decrease Unchanged	60% decrease Unchanged
Engelke et al ²⁵	Muscle	5	Ibuprofen	Oral	1200 mg	20% decrease	Unchanged
Addor et al ²¹	Muscle	5	Lysine acetylsalicylate	Oral	1800 mg	15% decrease	NA
Moppett et al ¹⁸	Skin	5 and 20	Ibuprofen	Oral	800 mg	Unchanged	NA
			Rofecoxib	Oral	25 mg	Unchanged	NA
Medow et al ²⁰	Skin	5	Ketorolac	Local (microdialysis)	10 mM*	75% increase	150% increase

Percent increase or decrease presented as rounded approximations for legibility.

*Drug concentration in microdialysis fluid.

AUC, area under curve; NA, not available.

reach peak plasma level after a single oral dose, as shown in detail in pharmacokinetic data of healthy volunteers.³⁰ In these studies, furthermore, the concentrations obtained after intake of a 30-mg tablet remained for at least 6 hours above 400 µg/L (≈ 1 nM), a concentration shown to effectively block the vasoconstriction induced by U46619, a TP receptor agonist, in an ex vivo model.³² In addition, 2 studies showed the ability of a single oral dose of 10 mg S18886 to improve flow-mediated vasodilation when evaluated 2 hours after drug intake in patients with high cardiovascular risk and evidence of endothelial dysfunction.^{33,34} Taken together, these data make it very unlikely that our negative results could be explained by insufficient tissue concentration of the TP receptor antagonist.

If efficient tissue concentrations of S18886 were reached in our conditions, the negative results imply either that vasoconstrictor prostanoids did not in fact modulate the observed postischemic responses or that they did via pathways distinct from TP receptor signaling. The first possibility would run counter to the evidence, cited in the Introduction, that ischemia as short as carried out here markedly increases the production of TxA2 in human forearm. However, this evidence comes from mostly ancient studies having assessed thromboxane B2 (the stable metabolite of TxA2) with immunoassays^{26–29} and whose results could not be replicated with more specific methods based on gas chromatography–tandem mass spectrometry.³⁵ It remains that the previously mentioned marked augmentation of reactive hyperemia by the local blockade of COX (using microdialysis for drug administration)²⁰ is a strong argument in favor of a modulatory influence of vasoconstrictor prostanoids, at least in the skin. Such influence could be exerted by eicosanoids distinct from TxA2, able to stimulate non-TP receptors that would be insensitive to S18886. The family of prostanoid receptors comprises multiple members,¹³ of which not only the TP but also the EP1,³⁶ EP3,³⁷ and FP³⁸ types are able to mediate the contraction of vascular smooth muscle. Further work would be required to determine whether reactive hyperemia in human forearm tissue is modulated by eicosanoid ligands to these additional receptors, such as prostaglandin E2 (EP1 and EP3) or prostaglandin F2a (FP).¹³

CONCLUSIONS

Blockade of the TP receptor with S18886 did not affect the reactive hyperemia elicited by a short period of ischemia in the forearm muscle and skin of young healthy male humans. Because the importance of vasoconstrictive prostanoids increases with aging,³⁹ it remains to be determined whether a similar conclusion holds in older subjects. Further studies are anyway required to determine the pathways whereby COX products modulate postischemic vasodilation.

ACKNOWLEDGMENT

The authors thank Guy Berset for technical assistance.

REFERENCES

- Meredith IT, Currie KE, Anderson TJ, et al. Postischemic vasodilation in human forearm is dependent on endothelium-derived nitric oxide. *Am J Physiol*. 1996;270(pt 2):H1435–H1440.
- Kubli S, Waeber B, Dalle-Ave A, et al. Reproducibility of laser Doppler imaging of skin blood flow as a tool to assess endothelial function. *J Cardiovasc Pharmacol*. 2000;36:640–648.
- Tur E, Yosipovitch G, Bar-On Y. Skin reactive hyperemia in diabetic patients. A study by laser Doppler flowmetry. *Diabetes Care*. 1991;14:958–962.
- Hirooka Y, Imaizumi T, Tagawa T, et al. Effects of L-arginine on impaired acetylcholine-induced and ischemic vasodilation of the forearm in patients with heart failure. *Circulation*. 1994;90:658–668.
- Wu TC, Chen YH, Chen JW, et al. Impaired forearm reactive hyperemia is related to late restenosis after coronary stenting. *Am J Cardiol*. 2000;85:1071–1076.
- Skrha J, Prazny M, Haas T, et al. Comparison of laser-Doppler flowmetry with biochemical indicators of endothelial dysfunction related to early microangiopathy in Type 1 diabetic patients. *J Diabetes Complications*. 2001;15:234–240.
- Sanada M, Higashi Y, Nakagawa K, et al. Hormone replacement effects on endothelial function measured in the forearm resistance artery in normocholesterolemic and hypercholesterolemic postmenopausal women. *J Clin Endocrinol Metab*. 2002;87:4634–4641.
- Vuilleumier P, Decosterd D, Maillard M, et al. Postischemic forearm skin reactive hyperemia is related to cardiovascular risk factors in a healthy female population. *J Hypertens*. 2002;20:1753–1757.
- Binggeli C, Spieker LE, Corti R, et al. Statins enhance postischemic hyperemia in the skin circulation of hypercholesterolemic patients: a monitoring test of endothelial dysfunction for clinical practice? *J Am Coll Cardiol*. 2003;42:71–77.

10. Mitchell GF, Vita JA, Larson MG, et al. Cross-sectional relations of peripheral microvascular function, cardiovascular disease risk factors, and aortic stiffness: the Framingham Heart Study. *Circulation*. 2005; 112:3722–3728.
11. Ishibashi Y, Takahashi N, Shimada T, et al. Short duration of reactive hyperemia in the forearm of subjects with multiple cardiovascular risk factors. *Circ J*. 2006;70:115–123.
12. Feletou M, Huang Y, Vanhoutte PM. Endothelium-mediated control of vascular tone: COX-1 and COX-2 products. *Br J Pharmacol*. 2011;164:894–912.
13. Woodward DF, Jones RL, Narumiya S. International Union of Basic and Clinical Pharmacology. LXXXIII: classification of prostanoid receptors, updating 15 years of progress. *Pharmacol Rev*. 2011;63:471–538.
14. Krumholz HM. What have we learnt from Viiox? *BMJ*. 2007;334:120123.
15. Fosbol EL, Kober L, Torp-Pedersen C, et al. Cardiovascular safety of non-steroidal anti-inflammatory drugs among healthy individuals. *Expert Opin Drug Saf*. 2010;9:893–903.
16. Carlsson I, Linde B, Wennmalm A. Arachidonic acid metabolism and regulation of blood flow: effect of indomethacin on cutaneous and subcutaneous reactive hyperaemia in humans. *Clin Physiol*. 1983;3:365–373.
17. Bisgaard H, Kristensen JK, Sondergaard J. A new technique for ranking vascular corticosteroid effects in humans using laser-Doppler velocimetry. *J Invest Dermatol*. 1986;86:275–278.
18. Moppett IK, Davies JA, Mahajan RP. Non-selective and cyclo-oxygenase-2-specific non-steroidal anti-inflammatory drugs impair the hyperaemic response of skin to brief axillary artery occlusion. *Br J Anaesth*. 2003;91:353–356.
19. Dalle-Ave A, Kubli S, Golay S, et al. Acetylcholine-induced vasodilation and reactive hyperemia are not affected by acute cyclo-oxygenase inhibition in human skin. *Microcirculation*. 2004;11:327–336.
20. Medow MS, Taneja I, Stewart JM. Cyclooxygenase and nitric oxide synthase dependence of cutaneous reactive hyperemia in humans. *Am J Physiol Heart Circ Physiol*. 2007;293:H425–H432.
21. Addor G, Delachaux A, Dischl B, et al. A comparative study of reactive hyperemia in human forearm skin and muscle. *Physiol Res*. 2008;57:685–692.
22. Kilbom A, Wennmalm A. Endogenous prostaglandins as local regulators of blood flow in man: effect of indomethacin on reactive and functional hyperaemia. *J Physiol*. 1976;257:109–121.
23. Carlsson I, Wennmalm A. Effect of different prostaglandin synthesis inhibitors on post-occlusive blood flow in human forearm. *Prostaglandins*. 1983;26:241–252.
24. Carlsson I, Sollevi A, Wennmalm A. The role of myogenic relaxation, adenosine and prostaglandins in human forearm reactive hyperaemia. *J Physiol*. 1987;389:147–161.
25. Engelke KA, Halliwill JR, Proctor DN, et al. Contribution of nitric oxide and prostaglandins to reactive hyperemia in human forearm. *J Appl Physiol*. 1996;81:1807–1814.
26. Chen LS, Ito T, Ogawa K, et al. Changes of plasma 6-keto-PGF1 alpha and thromboxane B2 levels and platelet aggregation after tourniquet ischemia on the upper limb in normal subjects and patients with ischemic heart disease. *Jpn Circ J*. 1982;46:651–656.
27. Mathieson MA, Dunham BM, Huval WV, et al. Ischemia of the limb stimulates thromboxane production and myocardial depression. *Surg Gynecol Obstet*. 1983;157:500–504.
28. Lelcuk S, Huval WV, Valeri CR, et al. Inhibition of ischemia-induced thromboxane synthesis in man. *J Trauma*. 1984;24:393–396.
29. Kirkpatrick UJ, Burrows G, Blann A, et al. Neutrophil activation and severity of tissue hypoxia during tourniquet-induced forearm ischemia in man. *Exp Mol Pathol*. 1999;65:111–120.
30. Gaussem P, Reny JL, Thalamos C, et al. The specific thromboxane receptor antagonist S18886: pharmacokinetic and pharmacodynamic studies. *J Thromb Haemost*. 2005;3:1437–1445.
31. Siller-Matula JM, Krumphuber J, Jilma B. Pharmacokinetic, pharmacodynamic and clinical profile of novel antiplatelet drugs targeting vascular diseases. *Br J Pharmacol*. 2010;159:502–517.
32. Michel F, Simonet S, Vayssettes-Courchay C, et al. Altered TP receptor function in isolated, perfused kidneys of nondiabetic and diabetic ApoE-deficient mice. *Am J Physiol Renal Physiol*. 2008;294:F120–F129.
33. Belhassen L, Pelle G, Dubois-Rande JL, et al. Improved endothelial function by the thromboxane A2 receptor antagonist S 18886 in patients with coronary artery disease treated with aspirin. *J Am Coll Cardiol*. 2003;41:1198–1204.
34. Lesault PF, Boyer L, Pelle G, et al. Daily administration of the TP receptor antagonist terutroban improved endothelial function in high-cardiovascular-risk patients with atherosclerosis. *Br J Clin Pharmacol*. 2011;71:844–851.
35. Costa F, Sultur P, Angel M, et al. Intravascular source of adenosine during forearm ischemia in humans: implications for reactive hyperemia. *Hypertension*. 1999;33:1453–1457.
36. Rutkai I, Feher A, Erdei N, et al. Activation of prostaglandin E2 EP1 receptor increases arteriolar tone and blood pressure in mice with type 2 diabetes. *Cardiovasc Res*. 2009;83:148–154.
37. van Rodijnen WF, Korstjens IJ, Legerste N, et al. Direct vasoconstrictor effect of prostaglandin E2 on renal interlobular arteries: role of the EP3 receptor. *Am J Physiol Renal Physiol*. 2007;292:F1094–F1101.
38. Yu Y, Lucitt MB, Stubbe J, et al. Prostaglandin F2alpha elevates blood pressure and promotes atherosclerosis. *Proc Natl Acad Sci U S A*. 2009; 106:7985–7990.
39. Feletou M, Huang Y, Vanhoutte PM. Vasoconstrictor prostanoids. *Pflugers Arch*. 2010;459:941–950.