FLUID of artificial blisters from erythromelalgic skin areas in primary thrombocythaemia contained a high amount of prostaglandin-E-like activity. Dazoxiben did not alleviate the erythromelalgia in patients with primary thrombocythaemia despite complete inhibition of platelet malondialdehyde and thromboxane B₂ synthesis and no inhibition of prostaglandin-E-like material. During a 10-day dazoxiben treatment period, persistent erythromelalgia was associated with a significant shortened mean platelet life span of 3.2 days. During subsequent treatment with low dose acetylsalicylic acid daily complete relief of erythromelalgia was associated with inhibition of platelet prostaglandin endoperoxide production and correction of platelet mean life span to normal, 7.9 days. These observations indicate that prostaglandin E₂, or another prostaglandin endoperoxide metabolite, is involved in the pathogenesis of erythromelalgia. The presented study does not give one single clue as to the origin (platelet, vessel wall or other) of the prostanoid, but very likely originates from platelets because a very low dose of acetylsalicylic acid (250 to 500 mg every other day), which irreversibly inhibits platelet cyclooxygenase, is highly effective in the prevention of erythromelalgia in thrombocythaemia.

Key words: Aspirin, Cyclooxygenase, Dazoxiben, Erythromelalgia, Platelets, Prostaglandins, Thrombocythaemia, Thromboxane

Prostaglandin cyclooxygenase products but not thromboxane A₂ are involved in the pathogenesis of erythromelalgia in thrombocythaemia

J. J. Michiels^{1,CA} and F. J. Zijlstra²

¹Department of Haematology, University Hospital Dijkzigt Molewaterplein 40, 3015 GD, and

²Institute of Pharmacology, Erasmus University Medical School, Rotterdam, the Netherlands

^{CA} Corresponding Author

Introduction

Erythromelalgia is characterized by warm, red, congested extremities and painful burning sensations. In what appears to be the antithesis of Raynaud's disease, warmth intensifies the discomfort and cold provides relief. Acroparesthesias e.g. tingling, pins and needles sensations, and numbness in the toes and fingers usually precede the disabling and burning distress. Erythromelalgia may lead to painful acrocyanosis and peripheral gangrene.¹

In previous studies we demonstrated that erythromelalgia is causally related to thrombocythaemia and results from platelet mediated inflammation and microvascular changes (Fig. 1).^{1–3} The histopathological vascular changes are confined to arterioles and characterized by aspecific inflammation, fibromuscular intimal proliferation and occlusive thrombi in the absence of pre-existing vascular disease.⁴ Both clinical signs and vascular lesions completely disappear by treatment with the platelet inhibiting drugs acetylsalicylic acid (ASA) and indomethacin, which inhibits platelet aggregation by inactivation of platelet cyclooxygenase activity.

To obtain more information of the pathophysiologic mechanism of erythromelalgia, prostaglandin

activity in fluid from artificial blisters from erythromelalgic areas was investigated. Subsequently the authors evaluated the effects of dazoxiben, a selective inhibitor of thromboxane synthetase activity,⁵ on erythromelalgia, platelet kinetics and prostaglandin synthesis in patients with primary thrombocythaemia. Evidence is presented

FIG. 1. Pathophysiologic pathways in the genesis of platelet-mediated erythromelalgia and acrocyanotic ischaemia in thrombocythaemia.

that platelet prostaglandin E_2 or another prostaglandin endoperoxide metabolite is involved in the pathogenesis of erythromelalgia in primary thrombocythaemia.

Methods

Clinical and haematological data were obtained routinely. Thermography was carried out with a Bofors Mark II (Karls Koga, Sweden) camera, that registers the skin temperature indirectly. The skin surface temperature was compared with a reference source of fixed temperature.

Artificial dermal blisters were produced by a suction blister device according to Kristella,⁶ which was connected to the central suction unit of the hospital. The suction pressure was adjusted to 100 mmHg. The suction cups were cleaned with 70% alcohol and placed on the skin. Within a few minutes the pressure was decreased slowly to -200 mmHg. Blisters of 3 mm developed after 1 to 2 h. Blisters were aspirated using a thin needle and syringe.

Prostaglandin-like material was extracted from blister fluid according to the method of Unger *et al.*⁷ After resuspending the direct extracts in saline (NaCl 0.9%), PGE-like material was assayed against authentic PGE₂ (Upjohn Co., Kalamazoo, USA) on the isolated rat stomach strip, using the oil-bath technique of Ferreira and de Souza Costa.⁸ Both standard and test prostaglandins were injected in 10 μ l volumes directly into the Krebs' solution superfusing the tissue.⁹ All values for PGE-like material were expressed as ng/ml blister fluid.

Malondialdehyde production by arachidonic stimulated platelets in platelet-rich plasma was measured according to Smith *et al.*¹⁰ Plasma thromboxane B₂ (TxB₂) and prostaglandin E₂ (PGE₂) were measured in 10 ml peripheral venous blood samples collected under resting conditions in polypropylene tubes, containing 20 μ l of heparin (500 μ g/ml thromboliquine, Organon, the Netherlands) and 50 μ l indomethacin (0.1 mg/ml in 0.1 M phosphate buffer, pH 8.0). Blood samples were centrifuged immediately at 1400 $\times g$ for 10 min and the plasma stored at -20°C until assay. Two ml of plasma was applied to a Sep-Pak C₁₈ cartridge (Water Ass). The prostaglandin-like compounds were eluted with 2 ml of absolute ethanol, and 200 μ l aliquots dried under a stream of nitrogen at 40°C in a radioimmunoassay tube, then redissolved in assay buffer. Antibody for TxB₂ and PGE₂ were obtained from L'Institut Pasteur (Paris, France), ³H-TxB₂ and ³H-PGE₂ from New England Nuclear (Boston, USA) and standards of TxB₂ and PGE₂ from Sigma. Normal values for TxB₂ and PGE₂ were obtained from blood samples taken from controls (aged 22-78 years) on two occasions.

Platelet survival studies were performed with sodium ⁵¹Cr-chromate labelled autologous platelets.¹¹ The mean survival of platelets was calculated according to the multiple hit model, as recommended by the International Committee for Standardization in Haematology.¹²

Results

Clinical and haematological data of three patients with primary thrombocythaemia at the time of study are summarized in Table 1. Thermographic documentation of erythromelalgia in the left upper leg and the sole of the right foot in Case 1 is shown in Fig. 2. The skin surface temperature exceeded 31°C at places of red painful erythromelalgic hot

Table 1. Pertinent data at time of study

Case	Age	Sex	Platelets ($\times 10^9/l$)	[Presenting symptoms]
1	45	M	750	Disabling burning pain and red swelling of right forefoot sole and burning painful red spots in the skin of the left upper leg as shown in Fig. 2
2	73	M	1715	Burning pain and redness in toes and forefoot sole
3	65	M	930	Burning pain in red-bluish big toe, forefoot sole and lateral edge of the right foot

FIG. 2. Thermographic documentation of erythromelalgia in Case 1. (A) The skin surface temperature of painful red congested areas in the skin of the left upper leg and in the right forefoot sole exceed 31°C compared with that of the corresponding contralateral skin. (B) Complete relief of erythromelalgia 2 days after treatment with acetylsalicylic acid (500 mg per day) is associated with disappearance of hot spots on isothermograms.

Table 2. Prostaglandin E-like (PGE) activity in fluid of artificial blisters from skin areas with and without erythromelalgia in Patient 1 with primary thrombocythaemia as shown in Fig. 1

Volume of blister fluid (ml)	PGE (ng/ml)	Erythromelalgia
1.19	5.40	Present
1.29	6.53	Present
1.02	0.80	Absent
1.35	0.99	Absent*

*Erythromelalgic skin area during treatment with acetylsalicylic acid for 2 weeks.

spots, which completely disappeared by curative treatment with aspirin.

Prostaglandin measurements in fluid of artificial blisters from erythromelalgic areas in the left upper leg in Patient 1 with primary thrombocythaemia are shown in Table 2. PGE-like activities in blister fluid from skin areas with active erythromelalgia measured on two different occasions are evidently higher as compared with the value in blister fluid from skin areas without erythromelalgia. PGE-like activity in blister fluid from erythromelalgic areas during treatment with ASA declined to the level of the symptom-free skin area (Table 2).

The effects of platelet inhibiting drugs on erythromelalgia in typical cases of primary thrombocythaemia are shown in Fig. 3. Relief of erythromelalgic pain from one oral dose of ASA

Table 3. The effect of dazoxiben (200 mg every 6 h for 5 days) on platelet malondialdehyde (MDA) synthesis in Patient 2 who suffered from primary thrombocythaemia and erythromelalgia

Day	MDA (nmol/10 ⁹ platelets)		
	Pretreatment value	2 h	4 h
1	14.3	3.5	—
2		1.98	4.81
3		1.25	5.09
4		1.98	4.43
5		1.89	5.51

*Time lapse after intake of dazoxiben.

(500 mg) lasted 3 to 4 days, but from one oral dose of indomethacin (75 mg) less than 24 h. The analgesic effect of these drugs is in accordance with the length of inhibition of platelet malondialdehyde (MDA) production by arachidonic acid stimulated platelets in platelet-rich plasma (Fig. 3). Continued indomethacin (25 mg 3 times a day) relieved the erythromelalgia and inhibited MDA, and both reappeared within 24 h after discontinuation of the drug (Fig. 3).

Dipyridamole, 400 mg, sulphinpyrazon 800 mg, sodium salicylate 1500 mg (Fig. 3) and ticlopidine, 1000 mg (data not shown) for 4 days did not alleviate the erythromelalgic symptoms, but also had no effect on platelet MDA production (Fig. 3).

Treatment of Patient 2, who suffered from primary thrombocythaemia and erythromelalgia, with dazoxiben (200 mg orally every 6 h for 5 days) resulted in incomplete inhibition of platelet MDA synthesis of 90% at 2 h and of 75% at 4 h after each ingested dose of the drug (Table 3).

Treatment of Patient 3, who suffered from primary thrombocythaemia and erythromelalgia, with dazoxiben (400 mg every 6 h for 10 days) resulted in a nearly complete inhibition of MDA synthesis and complete inhibition of TxB₂ formation, but no inhibition of PGE₂ synthesis in exposed platelets (Table 4).

Dazoxiben treatment (400 mg 4 times a day for 10 days) did not relieve erythromelalgia and was associated with a significant shortened mean platelet survival of 3.7 days, indicating platelet consumption. Subsequent treatment with one low dose of ASA 500 mg daily, resulted in prompt and complete relief of erythromelalgia, correction of platelet mean survival to normal (7.9 days) and a change of platelet disappearance curves from curvilinear to a linear pattern.

Discussion

Erythromelalgia in thrombocythaemia is a platelet mediated syndrome of arteriolar inflammation and thrombosis of usually acral areas (Fig. 1)

FIG. 3. The effect of aspirin (acetylsalicylic acid), indomethacin, dipyridamol, sulphinpyrazone and sodium salicylate on erythromelalgia and on platelet cyclooxygenase activity, as measured by malondialdehyde (MDA) production by arachidonic acid stimulated platelets in platelet-rich plasma of patients showing primary thrombocythaemia.

Table 4. The effect of dazoxiben (400 mg every 6 h for 10 days) on platelet malondialdehyde (MDA) synthesis, plasma thromboxane B₂ (TxB₂) and prostaglandin E₂ (PGE₂) levels in Patient 3 who suffered from primary thrombocythaemia and erythromelalgia

Day	MDA (nmol per 10 ⁹ platelets)			TxB ₂ (pg/ml)			PGE ₂ (pg/ml)		
	C*	2 h**	4 h	C	2 h	4 h	C	2 h	4 h
1	20.1	2.0	3.06	1667	49	75	95	115	75
3		1.7	1.9		<15	<15		98	—
9		0.2	—		<15	<15		85	68

*Pretreatment value.

**Time lapse in hours after intake of dazoxiben.

and may occur in the skin of the leg simulating superficial thrombophlebitis.² Platelet consumption is evidently increased during active erythromelalgia.^{13,14} The drugs ASA and indomethacin, which inhibit platelet cyclooxygenase activity,¹⁵⁻¹⁸ induce complete relief of the erythromelalgic symptoms and recovery of the ischaemic circulation disturbances. The diagnostic long-lasting effect of a single low dose of ASA on erythromelalgia can readily be explained by its irreversible inhibition of platelet cyclooxygenase activity. One single dose of indomethacin improves erythromelalgia for less than 24 h, which corresponded with its reversible inhibition of platelet cyclooxygenase activity. In contrast, sodium salicylate and the platelet inhibiting drugs sulphinpyrazone, dipyridamole, ticlopidine and dazoxiben neither inhibit platelet cyclooxygenase activity^{19,20} nor alleviate erythromelalgia.² Curative treatment of erythromelalgia with ASA (500 mg per day) resulted in a significant increase of shortened platelet survival to normal.^{13,14} In contrast, dazoxiben neither affected erythromelalgia nor corrected the shortened platelet survival. These data are consistent with the concept that prostaglandin cyclooxygenase products but not thromboxane A₂ are necessary for the development of erythromelalgia.

Evidence of intravascular activation, secretion and aggregation of hypersensitive platelets in thrombocythaemia, taking place at high shear rate conditions in the end-arterial microvasculature, stems from the histopathological demonstration of fibromuscular intimal proliferation and thrombotic occlusions of arterioles and small arteries in skin areas of active erythromelalgia (Fig. 1).¹⁻⁴ The release of platelet derived growth factor during secretion and aggregation of platelets is supposed to account for the fibromuscular intimal proliferation. The release and activation of vaso-active substances, e.g. prostaglandins, during platelet mediated processes in thrombocythaemia may be responsible for the inflammatory symptoms of erythromelalgia (Fig. 1).² This assumption is supported by the finding of increased PGE-like

activity in blister fluid from erythromelalgic areas (Table 2). The presented study does not give one single clue as to the origin (platelet, vessel wall or other) of the prostanoid, but very likely originates from platelets, because a very low dose of ASA (250 to 500 mg every other day) is highly effective in the prevention of erythromelalgia—a most disabling condition. It is thought that cyclooxygenase activity in the platelets lacking nuclei is irreversibly inhibited by ASA, whereas the production of prostacyclin, which has an antiaggregation effect, is much less affected in the endothelial cells containing nuclei for protein synthesis of cyclooxygenase.^{21,22} Evidence exists that prostaglandin endoperoxides are important mediators of inflammation.^{23,24} Therefore, the presented clinical and experimental observations indicate that not thromboxane A₂, but its direct precursors, the prostaglandin endoperoxides or another prostanoid, prostaglandin E₂ in particular, are responsible for the pain and inflammation associated with erythromelalgia in primary thrombocythaemia.

References

- Michiels JJ. Erythromelalgia and thrombocythaemia. PhD Thesis, 1981; Rotterdam.
- Michiels JJ, Abels J, Steketee J, van Vliet HHDM, Vuzevski VD. Erythromelalgia caused by platelet-mediated arteriolar inflammation and thrombosis in thrombocythemia. *Ann Int Med* 1985; **102**: 466–471.
- Michiels JJ, van Joost T. Erythromelalgia and thrombocythemia: a causal relation. *J Amer Acad Dermatol* 1990; **22**: 107–111.
- Michiels JJ, Ten Kate FWJ, Vuzevski VD, Abels J. Histopathology of erythromelalgia in thrombocythaemia. *Histopathology* 1984; **8**: 669–678.
- Dale J, Thaulow E, Myhre E, Parry J. The effect of a thromboxane synthetase inhibitor, Dazoxiben, and acetyl-salicylic acid on platelet function and prostaglandin metabolism. *Thromb Haemostas* 1983; **50**: 703–705.
- Kristella U. Suction blister device for separation of viable epidermis from dermis. *J Invest Dermatol* 1968; **50**: 129–132.
- Unger WG, Stamford IF, Bennett A. Extraction of prostaglandins from human blood. *Nature* 1971; **233**: 336–337.
- Ferreira H, DeSouza Costa E. A laminar superfusion technique with much increased sensitivity for the detection of smooth muscle stimulating substances. *Eur J Pharmacol* 1976; **39**: 377–381.
- Bult H, Bonta IL. Prostaglandin endoperoxides, serotonin and the superfused rabbit aorta. *Agents and Actions* 1976; **6**: 712–720.
- Smith JB, Ingemann CM, Silver MJ. Malondialdehyde formation as an indicator of prostaglandin production by human platelets. *J Lab Clin Med* 1976; **88**: 167–172.
- International Committee for Standardization in Hematology. Recommended methods for radioisotope survival studies. *Blood* 1977; **50**: 1137–1144.
- International Committee for Standardization in Hematology. Recommended method for platelet survival studies. *J Nucl Med* 1988; **29**: 564–566.

13. Michiels JJ, Lindemans J, van Vliet HHDM, Abels J. Survival kinetics of platelets in thrombocythaemia related to erythromelalgia. *Brit J Haematol* 1982; **50**: 691.
14. Michiels JJ. Platelet mediated inflammation and thrombosis in thrombocythemia. *Blood* 1990; **76** (Suppl 1): 515.
15. Roth GJ, Majerus PhW. The mechanism of the effect of aspirin on human platelets. *J Clin Invest* 1972; **9**: 97-103.
16. Burch JW, Brenziger ML, Stanford M, Majerus PhW. Sensitivity of fatty acid cyclooxygenase from human aorta to acetylation by aspirin. *Proc Natl Acad Sci USA* 1978; **75**: 5181-5184.
17. Crook D, Collins AJ. Comparison of effects of aspirin and indomethacin on human platelet prostaglandin synthetase. *Ann Rheum Dis* 1977; **36**: 459-463.
18. Fitzgerald GA. Dipyridamole. *N Engl J Med* 1987; **316**: 1247-1257.
19. Moncada S, Korb J. Dipyridamole and other phosphodiesterase inhibitors act as antithrombotic agents by potentiating endogenous prostaglandin metabolism. *Lancet* 1978; **i**: 1286-1289.
20. Lagarde M, Ghazi I, Dochavanne, M. Effects of ticlopedine on platelet prostaglandin metabolism. *Prostaglandins Med* 1979; **2**: 433-440.
21. Moncada S, Vane JR. Arachidonic acid metabolites and the interactions between platelets and blood-vessel walls. *N Engl J Med* 1979; **300**: 1142-1147.
22. Kallmann R, Nieuwenhuis HK, de Groot PG, van Gijn J, Sixma JJ. Effects of low dose aspirin, 10 mg and 30 mg daily, on bleeding time, thromboxane production and 6-keto-PGF_{1α} excretion in healthy subjects. *Thromb Res* 1987; **45**: 355-361.
23. Kuehl FA, Humes JL, Egan EA. Role of prostaglandin endoperoxide in inflammatory processes. *Nature* 1977; **265**: 170-173.
24. Vane JR. Prostaglandins as mediators of inflammation. *Adv Prost Thromb Res* 1976; **2**: 791-801.

**Received 23 June 1993;
accepted 12 August 1993**