The Prostaglandin E1 Analog Misoprostol Reduces Symptoms and Microvascular Arteriovenous Shunting in Erythromelalgia – A Double-Blind, Crossover, Placebo-Compared Study

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Abstract

Based on previous experience with parenteral prostanoids, we studied the effect of misoprostol treatment, an orally administered prostaglandin E1 analog, in patients with erythromelalgia. Treatment with placebo was followed by treatment with misoprostol (0.4–0.8 mg per d), both for 6 wk. The patients (n=21) and a study nurse who administered the trial were blinded. The endpoints were change in pain and need for cooling and global assessment of the treatment. Following central body heat provocation, global skin perfusion, capillary morphology, and change in pain were also recorded before and after each treatment period. Results were compared with data from healthy control subjects (n=11) that did not undergo treatment. Clinical safety and tolerability evaluation included physical examinations, clinical laboratory tests, and monitoring of adverse events. All clinical outcome measures were significantly better after treatment with misoprostol ($p<0.01$) as compared with placebo treatment and after a 3-mo follow-up without treatment. The heat-induced increase in global perfusion after misoprostol treatment was similar to the control group and significantly lower when compared with baseline ($p<0.01$) and placebo treatment ($p<0.05$), respectively. This study demonstrates that misoprostol is clinically superior to placebo in patients with erythromelalgia. The results of the perfusion studies may imply that the mechanism of action of the beneficial effect of misoprostol is reduced microvascular arteriovenous shunting in affected skin.

Keywords: arteriovenous shunting, clinical trial, microcirculation, prostanoids
Abbreviations: AV, arteriovenous; EM, erythromelalgia

Erythromelalgia (EM) is a rare condition characterized by red, hot, and painful extremities. Local skin cooling provides relief. Warmth, exercise, dependency of the extremity, and tight shoes and gloves intensify the discomfort (Thompson et al, 1979). The condition may be primary or secondary to another disease (Smith and Allen, 1938).

Numerous therapies have been used with varied success, but no firm conclusions of the treatment effects can be drawn from the published literature (Cohen, 2000;Davis and Rooke, 2002;Mørk and Kvernebo, 2002). Management of the patients includes information about support groups, teaching non-harmful cooling techniques, and avoidance of precipitating or exacerbating factors (exercise, increased ambient heat). Underlying disease (secondary EM) or complications to EM (edema, maceration, dermatitis) should be corrected.

The pathogenesis of EM has been debated. Over the most recent years, evidence for microvascular arteriovenous (AV) shunting in affected skin as the common final path for the development of symptoms has been growing (Kvernebo, 1998;Mørk et al, 2000;2002a). The hypothesis postulates that available blood is maldistributed in the skin. An insufficient proportion of blood is directed through nutritional capillaries, leading to skin hypoxia, while a large proportion of the blood is shunted through microvascular anatomical or functional AV anastomoses. The skin hypoxia induces arteriolar dilatation resulting in increased, but still

maldistributed, skin perfusion, which gives rise to in elevated skin temperature, accelerated metabolism, and a further deterioration of skin nutrition. A vicious cycle is created (Kvernebo, 1998). This common final path of the pathogenesis is probably triggered by a number of mechanisms related to hemorheological problems, defects in prostaglandin metabolism, or sympathetic or endothelial function (Jørgensen and Søndegaard, 1978;Drenth et al, 1996;van Genderen et al, 1996;Littleford et al, 1999a,b;Sandroni et al, 1999;Davis and Sandroni, 2002;Mørk et al, 2002b).

According to the shunt hypothesis, substances that alter skin blood flow distribution or improve hemorheological properties of the blood may improve skin oxygenation and induce relief. The beneficial effects of vasodilators like sodium nitroprusside have been reported (Özsoylu and Coskun, 1984). Aspirin, which inhibits thrombocyte aggregation, may promptly relieve EM symptoms secondary to blood dyscrasia. Parenteral prostaglandin E1 (PGE1), prostacyclins, or analogs have been described to have beneficial effects (Belch, 1992;Kvernebo, 1998). In a recently published pilot study, using infusion with the prostacyclin analog iloprost, we have for the first time in a double-blind, randomized study described reduced symptoms and sympathetic dysfunction in EM patients (Kalgaard et al, 2003).

The present clinical trial was designed to determine whether treatment with misoprostol, an oral PGE1 analog, leads to improvement in EM symptoms as compared with placebo treatment. The mean EM pain for 1 wk following treatment was chosen as the primary clinical endpoint, while the patients' need for cooling and the global response to the treatment were secondary variables. A physiological study of the effect of misoprostol on the distribution of skin perfusion was added to the clinical trial. In this study of pathogenesis, we tested the hypothesis that misoprostol redistributes skin perfusion in favor of nutritive perfusion in patients with EM.

Results

All the enrolled patients received intended treatment, but one was not available at the time of follow-up. The changes in pain and cooling scores were calculated by subtracting the scores of the last week of the actual period minus the last week of the previous period. The change was significantly better for both parameters during treatment with misoprostol with compared with placebo (p<0.01) and follow-up (p<0.01) *(Figure 1)*. There was a correlation between pain and cooling score values (r=0.8, p<0.01) when all available pairs of data were used. Based on global self-assessment, treatment with misoprostol was superior to placebo (p<0.01), but the improvement did not persist at follow-up *(Figure 1)*. No statistical difference was demonstrated for global assessment between placebo and follow-up. During study design, a reduction in pain score 20% was considered to be clinically significant. Using this definition of "success", 14 patients were responders on misoprostol, compared with four on placebo and none in the follow-up period (p<0.05). Fourteen patients also reported improvement in global assessment during misoprostol treatment when compared with baseline, significantly better as compared with placebo treatment (n=5, p<0.05).
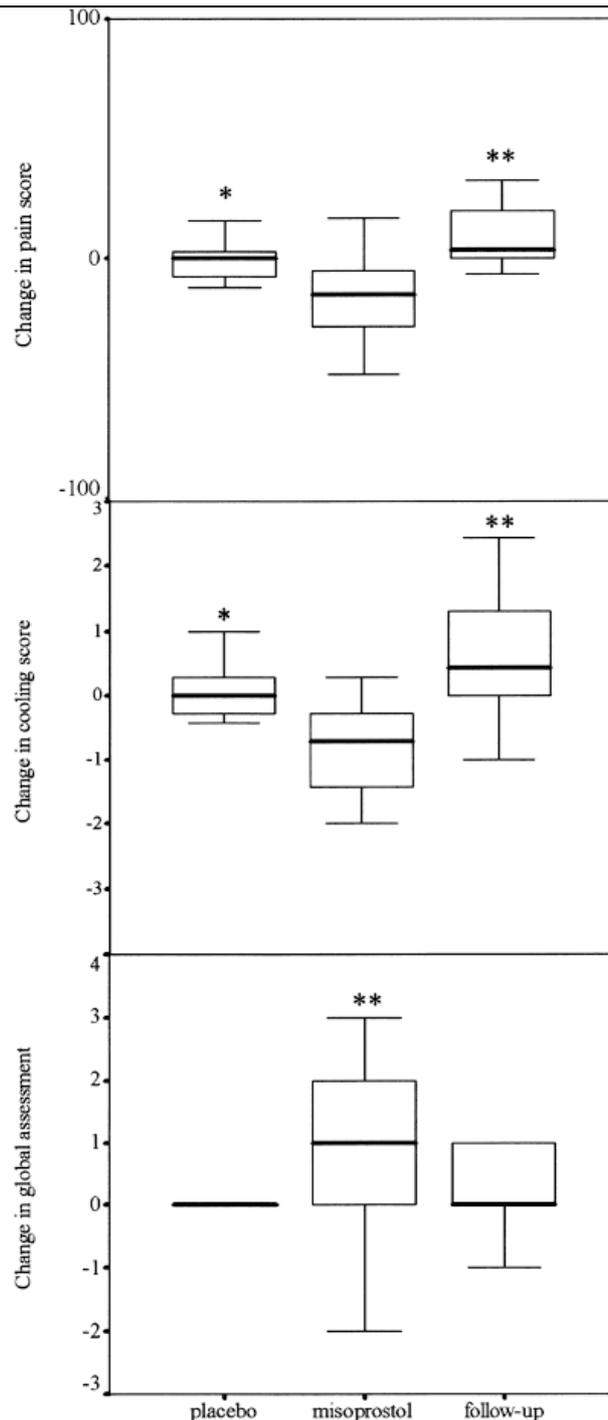
Figure1: Pain and cooling of affected skin were significantly reduced after treatment with misoprostol as compared to placebo, and a significant deterioration of pain was demonstrated after cessation of misoprostol. Global assessment of EM suffering was significantly improved after treatment with misoprostol as compared to treatment with placebo. Distribution of change in pain score (visual analogue scale, 0–100 mm), cooling score (Likert scale 0–8) and global assessment (8-point scale) as compared to baseline during each study period is presented as box plots, where the boxes show median with quartiles and the error bars 10/90 percentiles. *p=0.01, **p<0.01.

Reported clinical adverse events are summarized in *Table I*. All symptoms were within the known safety profile of misoprostol or considered unrelated to the study medication. The reactions considered to be possible or probably misoprostol-related were mild to moderate, dose-related, and resolved after discontinuation of the study medication. No clinically significant changes in laboratory test values or vital signs were recorded during the course of

the study. After treatment with placebo, three patients returned >30% of the dispensed medication, and four after treatment with misoprostol.

Table I. *Adverse events*

	Placebo (n)	Misoprostol (n)[a]
None	7	7
Gastrointestinal	8	12
Abdominal pain	0	7[3]
Nausea	1	3[3]
Loose stool/diarrhea	6	2[2]
Vomiting	0	2[3]
Flatulens	1	4[3]
Sore throat	5	2[1]
Headache	0	1[2]
Insomnia	1	0

(a) Relationship to misoprostol, **(1)** unlikely, **(2)** possibly, **(3)** probably.

For the physiological part of the study, two patients did not meet to the scheduled appointments after misoprostol treatment, and when rescheduled the microscope was no longer available. The number of asymptomatic patients after heat provocation was significantly higher after misoprostol (n=16) as compared with baseline (n=6, p<0.05) and after placebo treatment (n=7, p<0.05). The increase in pain score after central body heating was significantly lower after treatment with misoprostol as compared with baseline and placebo (p<0.01) Figure 2. No statistical difference was demonstrated between baseline and placebo.
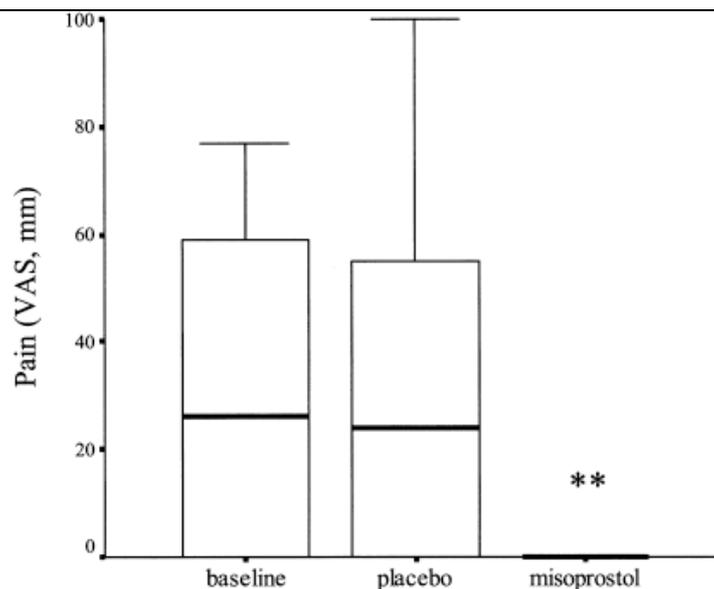

Figure 2: *Increase of pain score induced by heat provocation was significantly reduced after misoprostol treatment as compared to baseline and after treatment with placebo. Data are presented as box plots, where the boxes show median with quartiles and 10/90-percentiles. VAS=visual analogue scale, 0–100 mm. **p<0.01.*

On all occasions, patients and control subjects showed a significant increase in LDPI-assessed perfusion after central body warming (p<0.01) *(Table II)*. The pre-heating LDPI values did not differ between the groups. After misoprostol treatment, the increase in LDPI-assessed skin perfusion following heating did not differ from the response in the control group *(Figure 3)*. This is in contrast to assessments at baseline and after placebo treatment, where the increase in skin perfusion was significantly larger (p<0.01 and p<0.05) as compared with misoprostol and controls. Skin perfusion was reduced in one patient after heat provocation. This patient

had an EM attack with high skin temperature and high perfusion just prior to heat provocation.
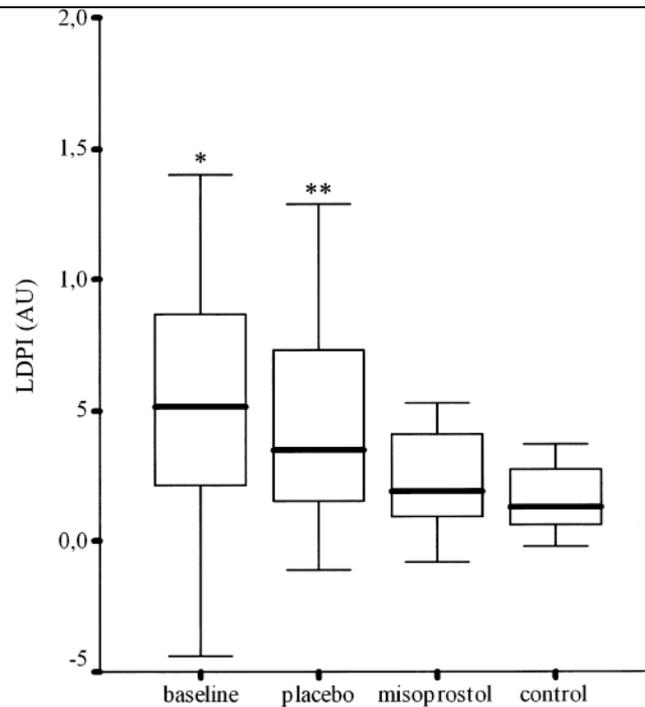

Figure 3: Increase in LDPI assessed skin perfusion induced by heat provocation was significantly lower in the control group and in EM patients after treatment with misoprostol as compared to baseline and placebo. Data are presented as box plots, where the boxes show median with quartiles and 10/90 percentiles. AU=arbitrary units. *p<0.05 as compared to control and misoprostol, **p<0.01 as compared to control and misoprostol.

Table II. EM severity (pain VAS, mm), skin temperature at first toe (°C), LDPI-assessed skin perfusion (AU[a]), number, and mutual distance (AU[a]) of skin capillaries in the field of view (1.7 mm^2) before and after heat provocation, respectively

	Baseline		Placebo		Misoprostol		Control	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Severity[b]	0 (0-55)	49 (0-100)[e]	0 (0-65)	27 (0-100)[e]	0 (0-66)	0 (0-82)[d]		
Temperature[b,c]	24.4 (21.1-34.6)	34.1 (25.7-36.4)[e]	24.2 (20.1-34.8)	33.0 (24.6-35.3)[e]	24.6 (21.3-35.2)	32.3 (22.9-35.7)[e]	23.3 (21.1-32.0)	30.2 (24.4-34.3)[e]
LDPI[b]	0.53 (0.32-1.93)	1.34 (0.44-2.88)[e]	0.47 (0.28-1.85)[f]	0.88 (0.34-2.26)[e]	0.40 (0.31-1.31)[f,g]	0.68 (0.31-1.84)[e]	0.51 (0.34-1.32)	0.78 (0.41-1.36)[e]
Capillary density[b]	87 (62-116)	77 (48-114)[e]	80 (53-129)[h]	57 (41-109)[e]	87 (54-109)	76 (56-108)[d]	93 (73-148)	98 (69-133)
Mutual distance[b]	40 (35-48)	42 (35-55)[e]	42 (33-51)[i]	48 (35-56)[e]	39 (36-51)	43 (35-49)[d]	39 (31-46)	37 (33-47)

(a) AU, arbitrary units. **(b)** Median with total range. **(c)** Toe temperature recorded during LDPI scanning. **(d)** p<0.05 **(e)** p<0.01 as compared with the the respective pre-heating values. **(f)** p<0.01 as compared with the pre-heating LDPI value at baseline. **(g)** p<0.05 as compared with the pre-heating LDPI value of healthy controls. **(h)** p<0.01 as compared with the pre-heating capillary density value for healthy controls. **(i)** p<0.05 as compared with the pre-heating mutual distance value for healthy controls.

After central body warming, capillary density was reduced in EM patients at baseline, after placebo and after misoprostol treatment, in spite of the significant increase in temperature and perfusion *(Table II)*. This was in contrast to findings in controls where capillary density was unchanged in response to heat provocation. The average distance between neighboring skin capillaries (mutual distance), an estimate of diffusion distances for oxygen and nutrients, increased in patients after warming, but not in controls. For both CAVM parameters, treatment with misoprostol tended to give results more similar to controls.

Retrospective subgroup analyses of demographic (age, gender) and clinical characteristics (age of onset and duration of EM, EM category, or baseline severity, responders versus non-responders) did not demonstrate significant differences for any clinical or physiological variables.

Discussion

The oral PGE1 analog misoprostol reduced EM symptoms significantly more than treatment with placebo, as determined by pain and cooling scores, and global assessment. The therapeutic effect had ceased 3 mo after discontinuation of misoprostol treatment. Furthermore, misoprostol reduced pain and hyperemia, induced by central body heating, as compared with patient recordings at baseline and after placebo treatment. To our knowledge, this is the first publication of a properly designed placebo-controlled clinical trial for the treatment of EM.

The main cause of the lack of clinical trials in EM patients is the low prevalence of the condition (Kvernebo, 1998). The present study was possible due to our systematic building up of a database of patients over a time period of 20 y. We applied wide inclusion and limited the number of exclusion criteria to obtain a sufficient number of patients. This resulted in a relatively heterogeneous study population with respect to severity and etiology, increasing the external validity of the results. The patients were, with one exception, classified as chronic cases, shown to have a stable course (Kalgaard et al, 1997). In this study rather small fluctuations in symptoms were recorded during enrolment and treatment with placebo. This stability is an important requirement for choosing a crossover design. Long-term remission (3 mo to 2 y) has previously been described after PGE1 infusions (Kvernebo, 1998). The non-randomized study design was chosen to eliminate a possible carry-over effect of misoprostol from the first to the second treatment.

The outcome measures had previously been found to be clinically relevant in the characterization of EM severity (Kalgaard et al, 1997,2003;Kvernebo, 1998). The VAS method is the most commonly used tool to quantify pain intensity, because it is easily administered, requires a minimum of explanation to respondents, and is a sensitive tool for the measurement of pain differences (Torrance et al, 2001). We reduced the effect of daily fluctuations in EM severity by applying mean values of daily recordings for 1 wk. To ensure stable environmental conditions during the study, the treatment period was in the wintertime with a stable cold climate in Norway (the mean temperature with monthly mean range in the treatment period was +0.1°C (-1.3°C–1.4°C). The follow-up evaluation was in May with mean temperature +11.6°C.

The dosage of misoprostol treatment was based on recommendations from the pharmaceutical company (Searle), Norwegian Medicines Control Authority, and pre-study beneficial experience from two patients with EM. A dose–response curve for patients with EM was not performed. The duration of the treatment period represents a compromise between previous experience with misoprostol treatment in EM, and the potential of disease instability and inadequate patient cooperation with a long-lasting intervention. The incidence of drug-related side effects was minimized by a stepwise dosage increase, transient dose reduction if necessary, and administration of the study medication immediately after meals. More frequent adverse events during treatment with misoprostol may potentially have biased the blinding of the study medication and reduced adherence to therapy. Intolerance to drug therapy did not cause any patient withdrawals during the treatment period.

The treatment effect of misoprostol is statistically superior to placebo. We consider this difference to be clinically relevant because of the size of the effect, the large number of

responders, and the significant correlation between the clinical outcome measures. At a 3-mo follow-up, no long-term effect of misoprostol could be demonstrated.

No constant effect of any treatment apart from local cooling has so far been documented. A limited number of patients is one explanation. The other is that EM patients are a heterogeneous group with respect to disease mechanisms. We have managed to collect a sufficient number of patients to draw statistical conclusions, but the low number makes retrospective subgroup analyses difficult. Those patients reporting placebo effects reported further improvement after treatment with misoprostol. One patient with thrombocythemia had EM symptoms for the first time just prior to the start of this study. Maximum EM severity was reached within 1 mo of placebo treatment and the patient was asymptomatic 1 wk prior to the stop of placebo. The remission persisted to the end of this study, consistent with acute EM. If patients with low adherence to treatment (return of >30% of dispensed tablets) and/or the patient with acute EM are eliminated, the clinical efficacy of misoprostol is strengthened versus placebo.

In previous studies we have used LDPI and CAVM to assess microvascular AV shunting as a pathogenetic mechanism in EM (Mørk et al, 2000;2002a). In the present therapeutic trial, we have assessed the effect of treatment using the same two techniques. The LDPI signal is dependent on capillary nutritive perfusion as well as blood flow in venous plexa and through AV shunts, global skin perfusion (Arildsson et al, 2000;Nilsson et al, 2003). The laser Doppler signal is influenced by skin thickness and color. In this study all patients were Caucasians and the measurements were performed at the same site. In a previous publication, we demonstrated that the flow increase in the feet of symptomatic EM patients following central body warming was higher as compared with asymptomatic patients and controls (Mørk et al, 2000). It was postulated that the cause was a disproportional increase in thermoregulatory skin perfusion through microvascular AV shunts. The EM patients in this study also had a significantly larger increase in global skin perfusion following warming at baseline and after placebo treatment, as compared with controls *(Figure 4)*. After misoprostol treatment, the EM patients experienced reduced pain after warming, and at the same time the flow increase was reduced to levels comparable to the control group. The simultaneous reduction of heat-induced pain and reduced hyperemia may imply reduced shunting as a mechanism of action for the beneficial effect of misoprostol. When nutritional capillaries are opened by the vasodilator, the EM symptoms are reduced or disappear due to improvement or elimination of skin hypoxia. The clinical effect of treatment is dependent on the capacity of the drug to redistribute the skin perfusion in favor of the capillaries. With higher levels of skin oxygen tension, the supplying arterioles constrict, with the consequence that the total skin perfusion decreases. As the global skin perfusion is maldistributed in favor of the shunt flow, the arteriolar constriction reduces skin temperature, skin metabolism and oxygen consumption, and the vicious cycle is broken.

	Pretreatment	Treatment				Posttreatment	
		Placebo		Misoprostol			
Tablets (n)	0	1x2	2x2	1x2	2x2	0	
Time (weeks)	-1	0	1	6	7	12	24
Clinical evaluation	↑	↑		↑		↑	↑
Physiological measurements	↑			↑		↑	

Figure 4: Study flow chart.

In a previous paper, EM patients who did not develop symptoms during heat provocation behaved like controls in respect to CAVM parameters. Patients who developed pain during warming reduced the skin capillary density (Mørk et al, 2002a). Also these findings gave

support to the microvascular AV hypothesis as a pathogenetic mechanism. The EM population in the present study was a mixture of patients with and without symptoms both before and after heat provocation. At baseline, six of 21 patients were asymptomatic after heat provocation, compared with 16 of 21 following misoprostol. In spite of the clinical effect and the LDPI findings, we still found a reduction in CAVM-assessed capillary density and an increase in mutual distance induced by warming *(Table II)*. Shunting therefore still takes place after misoprostol treatment, but may be below a critical level for inducing pain.

In conclusion, misoprostol treatment is statistically superior to treatment with placebo, and we consider the treatment difference to be clinically significant. The mechanism of the effect is probably reduction of microvascular AV shunting. The oral administration of the treatment is also of great value to the patients. The drug is relatively well tolerated, and we therefore recommend misoprostol as the first-line treatment of EM. In severe and resistant cases, parenteral treatment with prostanoids may be indicated. In future, larger randomized parallel group clinical trials are desired to document long-term treatment effects, and the study responses in subgroups of patients. Such studies will have to be undertaken on a multicenter basis.

Materials and Methods

Subject selection
Patients were recruited from our database of 106 EM cases systematically collected since 1983. Adult (ages 18–75 y), primary or secondary EM cases, with mild to severe symptoms (average cooling score 1 the week prior to inclusion, see Methods) localized to the feet, were assessed for eligibility (Kvernebo, 1998). Fertile females had a negative pregnancy test before enrolment, and were instructed to practise contraception during the study. Exclusion criteria included confounding concurrent diseases or drugs. Combining the current clinical and epidemiological information in our database (Kalgaard et al, 1997), 63 patients were found suitable for a questionnaire about EM symptoms for the last year. Twenty-one patients entered a screening procedure 1 wk prior to intervention, including medical history, physical examination, and laboratory evaluation, following a daily recording of current disease severity according to the cooling score (see Evaluation procedure). All patients enrolled in the study underwent intervention after signing a written informed consent form. For physiological evaluation of the effect of therapy, the patients were compared with values from 11 healthy controls. For logistic reasons, some control subjects were examined with one of two measuring techniques. The baseline demographics and clinical characteristics are given in *Table III*. Nine patients were classified as secondary EM, and comorbidities included connective tissue disease (n=3), myeloproliferative disease (n=2), diabetes mellitus (n=2), and sciatica (n=2). Nine patients had previously used aspirin, of whom one with EM secondary to polycythemia had a beneficial effect. They were instructed not to use aspirin during the course of the study. The Regional Committee of Research Ethics and the Norwegian Medicines Control Authority approved the study protocol. The Declaration of Helsinki Guidelines were followed.

Table III. *Demographic and clinical data of patients with erythromelalgia and healthy controls*

	EM	Control CAVM[a]	Control LDPI[b]
Age[c]	47.8 (21.2–60.6)	29.0 (24.3–75.2)	38.3 (24.3–75.2)
Female/male	14/7	4/7	9/2
Duration of EM[c]	11.2 (0.1–34.7)		
EM severity[c,d]	2.7 (1.0–7.0)		
No. of smokers	15	2	3
Cigarettes per [d]	15 (3–30)	10 (8–12)	10 (8–12)

(a) Computer-assisted video microscopy. (b) Laser doppler perfusion imaging. (c) Values given as median with total range. (d) Cooling score in the run-in period.

Study design

Based on an assumption on available patients, power considerations and previous experience with a possible carry-over effect of PGE1 in EM patients, we chose a double-blind, placebo-controlled, non-randomized, one-way crossover design *(Figure 4)*. A study nurse, unaware of the treatment sequence, administered and monitored the study medication and gave patients oral and written information throughout the study. The study nurse also instructed the patients in the scoring of outcome measures, handled, collected, checked and registered the patients' self-assessment forms, laboratory and adverse events reports, concomitant therapy and other procedures, and recorded vital signs (pulse and blood pressure). After a 1-wk run-in period, placebo followed by misoprostol 0.2 mg tablets were given, both for 6 wk. The placebo tablets were identical (size, form, and color) to the misoprostol tablets. The patients were instructed to take one tablet twice daily for the first week and, if tolerated, two tablets twice daily for the next 5 wk. The tablets should be taken immediately after a meal to reduce the chance of side effects. A questionnaire was sent to the patients at 3-mo follow-up. The control group was examined only once in the laboratory.

Evaluation procedure

The primary clinical efficacy measure was change in pain. The patients scored the pain intensity before and after interventions from the most affected foot for the last week on a 100 mm visual analog scale (VAS), where the anchors were marked "no EM pain" (0 mm) and "worst imaginable EM pain" (100 mm) by placing a perpendicular line somewhere on the non-graded line (Torrance et al, 2001). Secondary clinical outcome measures included change in cooling score and patients' global assessment of response to treatment. The cooling score was rated daily on an 8-point Likert (ordinal) scale during run-in and during each treatment period. Score 1 implies feeling uncomfortably warm without a need for cooling, and score 8 implies maximal burning pain with a continuous need for cooling or plexus/epidural anesthesia (Kalgaard et al, 1997). The scores are given as weekly averages. The global assessment (8-point scale: +4=cured, +3=greatly improved, +2=moderately improved, +1=slightly improved, 0=no change, -1=slightly decreased, -2= moderately decreased and -3=greatly decreased) of EM symptoms as compared with baseline was rated and at the end of weeks 6, 12, and 24 *(Figure 4)*. Safety monitoring included clinical evaluation and adverse event reports (weeks 6, 12, and 24). Laboratory evaluation (weeks 6 and 12) included hematology, liver and renal function tests, glucose, electrolytes, and urine stix. The principal investigator determined the potential relationship of the adverse events to the study medication as unrelated, possible, probably, and definitely. Compliance was assessed by the number of tablets returned. On three occasions *(Figure 1)*, pain score and physiological skin perfusion parameters (skin temperature, laser doppler perfusion imaging (LDPI), computer-assisted video microscopy (CAVM)) were recorded before and after provocation with central body heating.

Methods

Skin temperature was measured at the pulp of the first left toe using a digital handheld thermometer (Anritsu HFT-80 Meter, Japan). The principles governing measurements of microvascular perfusion by laser Doppler flowmetry have been described in detail elsewhere (Nilsson et al, 2003). In our study the skin microcirculation was examined with LDPI (PIM 1.0 Lisca Development AB, Linköping, Sweden), a technique employing multiple point recording for visualization of skin blood perfusion in terms of two-dimensional flow maps. LDPI offers a system for non-invasive, non-touch imaging of superficial microvascular blood perfusion and therefore prevents external mechanical sensory stimuli that may influence perfusion in the microvascular network under study. The complete system consists of a computer-controlled laser scanner and software for flow visualization. In microvascular applications the laser Doppler technique gives a semi-quantitative estimate of the perfusion. Data are expressed as flux or perfusion, defined as the product of the number and mean velocity of the blood cells in the measuring volume. The LDPI output is dependent on global skin perfusion, i.e., both nutritive capillary and AV shunt flow. The technique has previously been used to study shunt flow in EM patients (Mørk et al, 2000).

Combining modern microscopic techniques and information technology, a package able to facilitate the extraction of capillary parameters from microscopic skin images has been developed, analyzing capillary density (Zhong et al, 2000). The software can also calculate the mutual distance, which after creation of a triangulation map between visible capillaries gives the average length of the limbs of the triangles. The hardware of the microscopy system comprises a video capillary microscope and a PC with special software. The capillary microscope is a portable video microscope with a video signal control unit and a handheld mini-CCD camera (Microvision MV2100, Finlay Microvision, Warwickshire, UK). Magnification of the system was set to 200 to enable investigation of an ensemble of approximately 100 capillaries in a field of view of 1.7 mm^2. The color images captured by the system show capillary ensembles in which the capillaries appear as dark red spots with comma-like shapes. These spots are actually the apexes of the active capillary loops perpendicular to the skin surface. Changes in capillary density and mutual distance were used as parameters for nutritive perfusion. The technique has previously been used to support the hypothesis of AV shunting in EM (Mørk et al, 2002a).

Laboratory procedures

The subjects were instructed not to eat, drink coffee, tea or alcohol, or smoke for a minimum of 3 h before measurements. Twenty minutes prior to measurements, they were recumbent in a room with an ambient temperature of 23°C (22°C–24°C) without physical or psychological stress. In order to induce EM attacks, the subjects were covered with multiple duvets, except for the head, for 30 min. A metal cage separated the feet from the duvets. Skin temperature, skin perfusion levels, and current pain intensity (VAS) were recorded before and after central body heating. If pain was rated 0–10 mm on the VAS scale, the patients were considered asymptomatic. The measurement site for capillary microscopy was the terminal phalanx of the first toe, just proximal to the nail bed. In this region the capillary loops are perpendicular to the skin surface and the vasculature contains numerous AV shunts (Grant and Bland, 1931;Sherman, 1963). For LDPI, the measurement site was in the anterior section of the foot arch containing some, but fewer, AV shunts. Microscopic images were recorded before and after central body heating at tiny spots marked with black ink in the nail fold area. To make the skin more transparent and to minimize reflections from the surface, the measurement sites were prepared with immersion oil of room temperature just prior to measurements. The handheld video-microscopy unit was vertical to the skin surface and hardly touching it so as to prevent application of pressure to the underlying tissue. The intensity of the light source

was adjusted to maximize the visual contrast in the image. Captured images were stored in the internal database of the image capillary analysis program for later processing. The extent of the skin area to be imaged by laser Doppler was marked on the skin, so that the same area was scanned before and after heating. The distances of the measured areas to anatomical landmarks were recorded, so that the same area could be measured at baseline and after treatment with placebo and misoprostol. The distance between the scanner head and the skin was set to 10 cm in all measurements together with an image format of 2020 pixels, and resolution was set to "high" giving a morphological resolution level of approximately 0.4 mm. The patients rested in a supine position, with the test foot stabilized with pillows of sand to avoid gross movement artifacts.

Statistical analyses

It was calculated that a sample size of 20 patients would have 80% power to detect a difference of 20% in the primary efficacy variable at a 5% significance level. Values showed a skewed distribution, as assessed graphically and by the Kolmogorov-Smirnov test, and are given as medians with total range or box plots with quartiles and 10-, 90-percentiles. Efficacy and safety outcomes were analyzed using Friedman's test for more than two groups and the Wilcoxon signed ranks test for two groups. Fisher's, Kruskal-Wallis, and Mann-Whitney tests were used comparing different groups. Analyses were applied on the intention-to-treat population. If patients discontinued the study prematurely, missing data were estimated by carrying data forward from the previous visit. Differences were considered statistically significant if p values were 5%, after adjustments for multiple comparisons. All significance levels reported are two-tailed. Retrospective exploratory subgroup analyses were carried out according to clinically relevant combinations of baseline data, using Fisher's, Mann-Whitney, and Spearman ranks correlation tests. Analyses were performed using SPSS 10.0 (SPSS, Chicago, Illinois) and nQuery Advisor 2.0 (Statistical Solutions, Cork, Ireland) software.

References

1. Arildsson M, Asker CL, Salerud EG & Strömberg T. Skin capillary appearance and skin microvascular perfusion due to topical application of analgesia cream. Microvasc Res (2000) 59: 14–23.

2. Belch JJF. Erythromelalgia: A personal perspective. J Dermatol Treat (1992) 3: 153–158.

3. Cohen JS. Erythromelalgia: New theories and new therapies. J Am Acad Dermatol (2000) 43: 841–847.

4. Davis MD & Rooke T. Erythromelalgia. Curr Treat Options Cardiovasc Med (2002) 4: 207–222.

5. Davis MD & Sandroni P. Lidocaine patch for pain of erythromelalgia. Arch Dermatol (2002) 138: 17–19.

6. Drenth JPH, Vuzevski V, van Joost T, Casteelsvandaele M, Vermylen J & Michiels JJ. Cutaneous pathology in primary erythromelalgia. Am J Dermatol (1996) 18: 30–34.

7. Grant RT & Bland EF. Observations on arteriovenous anastomoses in human skin and in the bird's foot with special reference to the reaction to cold. Heart (1931) 15: 385–407.

8. Jørgensen HP & Søndegaard J. Pathogenesis of erythromelalgia. Arch Dermatol (1978) 114: 112–114.

9. Kalgaard OM, Seem E & Kvernebo K. Erythromelalgia: A clinical study of 87 cases. J Intern Med (1997) 242: 191–197.

10. Kalgaard OM, Mørk C & Kvernebo K. Prostacyclin reduces symptoms and reverses the sympathetic dysfunction in erythromelalgia in a double blind randomised pilot study. Acta Dermatol Venereol (2003) 83: 442–444.

11. Kvernebo K. Erythromelalgia—A disease caused by microvascular shunting. VASA (1998) 51 suppl: 1–39.

12. Littleford RC, Khan F & Belch JJF. Skin perfusion in patients with erythromelalgia. Eur J Clin Invest (1999a) 29: 588–593.

13. Littleford RC, Khan F & Belch JJF. Impaired skin vasomotor reflexes in patients with erythromelalgia. Clin Sci (1999b) 96: 507–512.

14. Mørk C, Asker CL, Salerud G & Kvernebo K. Microvascular arteriovenous shunting is a probable pathogenetic mechanism in erythromelalgia. J Invest Dermatol (2000) 114: 643–646.

15. Mørk C, Kalgaard OM & Kvernebo K. Impaired neurogenic control of skin perfusion in erythromelalgia. J Invest Dermatol (2002b) 118: 699–703.

16. Mørk C & Kvernebo K. Erythromelalgia—therapeutic strategies. In: Lebwohl M, Heymann WR, Berth-Jones J, Coulson I, edsTreatment of Skin Disease. Comprehensive Therapeutic Strategies (2002) St Louis, MO: Mosby p 212–214.

17. Mørk C, Kvernebo K, Asker CL & Salerud EG. Reduced skin capillary density during attacks of erythromelalgia implies arteriovenous shunting as pathogenetic mechanism. J Invest Dermatol (2002a) 119: 949–953.

18. Nilsson GE, Salerud EG, Strömberg T & Wårdell K. Laser doppler perfusion monitoring and imaging. In: Tal Vo Dinh, edBiomedical Photonics Handbook (2003) Boca Raton, FL: CRC Press p 1–24.

19. Özsoylu S & Coskun T. Sodium nitroprusside treatment in erythromelalgia. Eur J Paediatr (1984) 141: 185–187.

20. Sandroni P, Davis MDP, Harper CM, Jr, Rogers RS III, O'Fallon WM, Rooke TW & Low PA. Neurophysiologic and vascular studies in erythromelalgia: A retrospective analysis. J Clin Neuromuscular Dis (1999) 1: 57–63.

21. Sherman JL. Normal arteriovenous anastomoses. Medicine (Baltimore) (1963) 42: 247–267.

22. Smith LA & Allen EV. Erythermalgia (erythromelalgia) of the extremities. A syndrome characterised by redness, heat and pain. Am Heart J (1938) 16: 175–188.

23. Thompson GH, Hahn G & Rang M. Erythromelalgia. Clin Orthop (1979) 144: 249–254.

24. Torrance GW, Feeny D & Furlong W. Visual analog scales: Do they have a role in the measurement of preferences for health states? Med Decis Making (2001) 21: 329–334.

25. Van Genderen PJ, Lucas IS, van Strik R, Vuzevski VD, Prins FJ, van Vliet HH & Michiels JJ. Erythromelalagia in essential thrombocythemia is characterized by platelet activation and endothelial cell damage but not by thrombin generation. Thromb Haemost (1996) 76: 333–338.

26. Zhong J, Asker CL & Salerud EG. Imaging, image processing and pattern analysis of skin capillary ensembles. Skin Res Technol (2000) 6: 45–57.

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