Sildenafil in the Treatment of Raynaud’s Phenomenon Resistant to Vasodilatory Therapy

Thesis submitted for the Degree of Doctor of Medicine to Saarland University faculty of medicine

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I. SUMMARY

**Background:** Vasodilatory therapy of Raynaud’s phenomenon represents a serious clinical problem as treatment remains often inefficient and may be not tolerated because of side effects.

**Aims:** To investigate the effects of sildenafil on symptoms and capillary perfusion in patients with Raynaud’s phenomenon.

**Methods:** We performed a double-blinded, placebo-controlled, fixed-dose, crossover study in 18 patients with symptomatic secondary Raynaud’s phenomenon resistant to vasodilatory therapy. Patients were treated with 50 mg sildenafil or placebo twice daily for 4 weeks. Symptoms were assessed by diary cards including a 10-point Raynaud’s Condition Score. Capillary flow velocity was measured in digital nailfold capillaries by means of a laser Doppler anemometer. Flow velocity was detected continuously at rest during 7 min in 3 capillaries under controlled conditions. In order to provide normal values we measured capillary flow velocity in studied 10 healthy young men on three consecutive days, thrice a day, at regular hours (11h00, 15h00, 18h00).

**Results:** Mean capillary flow velocity was 0.4 mm/s ± 0.01 in healthy subjects and 0.14 ± 0.02 mm/s in patients with Raynaud’s phenomenon (p=0.0001). Sildenafil increased capillary blood flow velocity in each individual patient independently of the randomization order, and mean capillary flow velocity of all patients quadrupled (0.56 ± 0.01 vs. 0.14 ± 0.02 mm/s, p = 0.0002). Capillary flow velocity during treatment with sildenafil even exceeded normal flow velocity in healthy subjects (0.56 ± 0.01 vs. 0.4 ± 0.01 mm/s, p=0.7372). On sildenafil the frequency of Raynaud attacks was significantly lower (32 ± 13 vs. 48 ± 16, p = 0.0049), the cumulative attack duration was significantly shorter (527 ± 123 vs. 1023 ± 227 min, p = 0.0036) and the mean Raynaud’s Condition Score was significantly lower (2.8 ± 0.5 vs. 2.0 ± 0.3 mm/s, p=0.0002). Two patients reported side effects leading to discontinuation of the study drug.
**Conclusions**: Sildenafil is a highly effective and well tolerated treatment in patients with Raynaud’s phenomenon. Treatment with sildenafil is able to normalize capillary flow velocity in Raynaud’s phenomenon. PDE-5 inhibition appears to be a promising new approach in patients with microcirculatory disorders.
II. INTRODUCTION

II.1 Raynaud’s phenomenon

Raynaud’s phenomenon is characterized by episodic digital ischemia, manifested clinically by the sequential development of digital blanching, cyanosis, and rubor of the fingers in response to cold and, in some patients, to emotional stress. The typical sequence is pallor caused by arterial constriction, consecutively cyanosis and finally reactive hyperemia (13,29,69) (Fig.1). The paroxysmal ischemia of the digits is due to constriction of the digital and palmar or plantar arteries; initial pallor indicates that vasoconstriction involves the small cutaneous vessels. Later the digital capillaries and venules become dilated and the slowed blood flow allows the hemoglobin to release more of its oxygen, producing cyanotic, cold digits. When vasoconstriction is relieved, blood flow increases greatly (reactive hyperemia), imparting a red color to the previously ischemic digits (Fig.1).
Figure 1 Pathophysiology of Raynaud’s phenomenon (29).
Raynaud’s phenomenon, present in 3% to 5% of the general population, may be secondary to an underlying disease or anatomic abnormality, but more common is the primary Raynaud’s phenomenon, which is of unknown etiology. Secondary Raynaud’s phenomenon may occur in occlusive arterial disease, collagen disease (especially scleroderma), after trauma, after gangrene from any cause, drug intoxication (ergot, methysergide), blood dyscrasias (cryopathies, cold hemagglutinins), and neurogenic lesions (4). Total and capillary fingertip blood flow is smaller in patients with Raynaud’s phenomenon than in normal subjects. With a cooling stimulus, patients show a significant decrease in fingertip capillary flow that is also not seen in normal subjects. Trophic changes may appear in progressive cases. Recurrent infections, blisters, and small areas of local cutaneous gangrene may appear on the fingertips, but gangrene of a whole digit is rare.

Raynaud concluded from his early studies (1862, 1874) that excessive sympathetic activity was responsible for the attacks, but Lewis found that the digital vessels were abnormally reactive to local cold. Since ischemic attacks can still be induced after sympathectomy (35) it may be speculated that there is a local fault in the blood vessels in Raynaud’s disease. Raynaud’s phenomenon often occurs in patients with primary pulmonary hypertension; this may reflect a neurohumoral abnormality that affects both the pulmonary and digital circulation.

There have been many attempts to treat patients with Raynaud’s phenomenon, including biofeedback, vasodilatory medication, microvascular surgery and sympathectomy. All these attempts are less effective and may have severe side effects. The basis of treatment in Raynaud’s phenomenon remains vasodilatory medication. However, this therapy may be not tolerated in appropriate dose in most patients. Recent case reports (31,42) suggest that sildenafil, approved for treatment of erectile dysfunction, may improve capillary blood flow velocity and vasospastic symptoms in patients with Raynaud’s phenomenon. This is the first prospective and controlled trial which evaluates the efficacy of sildenafil in the treatment of Raynaud’s phenomenon.
II.2 Historic background of capillaroscopy

Less than fifty years after the discovery of the capillaries in frogs by Malpighi in 1661, Boerhaave was probably the first person to examine microcirculation. Boerhaave, whose physiological theories are based mainly on the capillary structure of human body, concentrated his studies especially on the intravascular aggregation of the blood corpuscle. It was Purkinje who was able to observe the main capillary by means of a magnifying glass. In 1874 Hueter developed the capillary microscopy of the lower lip. For the first time, in 1911, Lombard applied Vaseline on the skin in order to facilitate capillary microscopy. Unna had shown that epidermis is made transparent by this oil. During his microscopic examinations, Lombard noticed the especial formation of the capillaries in the nailfold. Therefore, he can be called one of the founders of the periungual capillaroscopy. In 1916 Eugen Weiss adopting Lombard's method, but using a camera, documented his observations. Others in Germany and U.S.A followed in their footsteps, among them Jaensch, Jurgensen, Wess, Niekau, Parrissius and Heimberger (6). In America G.E. Brown described the mega capillaries in scleroderma and Freelander and coworkers made the first cinematographically photos (15). After World War 2, thanks to the work of Bloch, Harders, Heising, Davis and Landaw, whose photographic Atlas of Clinical Capillary Microscopy was published in 1966 (15), attention was drawn to capillaroscopy. Besides getting knowledge on the morphology of the capillaries, Bengt Fagrell was able to perform dynamic examination on the capillaries in 1972 (27). Capillaroscopy of the skin and nailfold is used in various fields of medicine for research on different diseases and their medicinal treatment, e.g. dermatological illnesses, vascular acrosyndromes, iatrogenous and toxic traumatologic sicknesses, arteriopathies, diabetes mellitus, connective tissue diseases, vasomotoric disorders, and hematological ailments.

The use of video technique has helped to improve capillarmicroscopy considerably in the last two decades. Whereas in the past all attempts were concentrated on the registration of the morphology of capillaries and their significance for various diseases, now the measuring of the dynamic processes is the focus of attention (47). Owing to the
importance of the capillary flow for the nutrition of the skin, the quantitative recording has great significance.

It is especially in the field of measuring procedures that the recent methodological progress has been made e.g. in the examination of retina vessels (57,58), in the use of fluorescence substances in various circulatory parts (8,44) and in the evaluation of capillaroscopy (27). However, the majority of measuring techniques depend on focus, contrast, and involuntary movement of the test person. Thickly calloused skin or various skin diseases can also have an adverse effect. In the nailfold, lower lip and nipples capillaries are lying parallel to the plane of the skin. In the majority of other organs the sub epidermal capillary extends at right angles to the plane of the skin. Different video capillaroscopic methods make measuring of the capillaries running parallel to the skin-plane possible e.g. frame-to-frame (56,5), flying spot (62,11) or cross-correlation (26). As they are non-invasive, the sensor and the blood flow do not have any interrelations. To display the blood flow, these methods use white light. The resolution of the white light is limited. Moreover, only a fraction of the white light can be used, so that a more powerful source of signal is advantageous.

The videocapillaroscopic system used in this study (CAM1) provides a laser beam and links the videocapillaroscopy to the Doppler technique (laser Doppler anemometry). By means of a microscopic lens system, a beam of laser Doppler is directed exactly in a single capillary and thus making measuring of blood flow in a defined field of view possible. The fundamental technique underlying the laser Doppler anemometry has been verified both in vivo (58,71,72), and in vitro (20).
II.3 Anatomic basics

Skin is composed of the epidermis and the dermis (Fig. 2). Below these layers lies the hypodermis. The outermost epidermis is made up of stratified squamous epithelium with an underlying basement membrane. It contains no blood vessels, and is nourished by diffusion from the dermis. Blood capillaries are found beneath the epidermis, and are linked to an arteriole and a venule. Arterial shunt vessels may bypass the network in ears, the nose and fingertips. The structure of skin vascularization consists of two plexus, a deep lying plexus and a superficial dermal subpapillary plexus (15,70) (Fig. 2). The deep dermal plexus consists of small to medium size arteries and veins. The superficial plexus comprises the skin capillaries that can be studied by nailfold capillary microscopy. These capillaries are the tiniest possible ramifications of the vessel-system. They represent the transition from arteries to veins and have an average diameter of about 11 μm.
Figure 1 Course of the microcirculation in the skin (70).
II.4 Microcirculation and capillary microscopic view

Microcirculation can be defined as blood circulation in the tiniest blood vessels. The function of microcirculation is mainly the maintenance of homeostasis and cell metabolism and carrying out many other exchange functions. Microcirculation includes terminal blood vessels, i.e. arterioles, capillaries, venules, arteriovenular anastomoses and the lymph capillaries (56). On the whole 5% of the blood circulation goes to the nourishment of the skin. Blood circulation of the skin also serves the thermoregulation. The skin flow and the extent of flow change display great regional distinctions. The greatest changes occur in the acral extremities: the blood flow of the fingers can sink to 1 ml.min⁻¹.dl⁻¹ in cold surroundings and rise to 100 ml.min⁻¹.dl⁻¹ in a warm environment. The number of perfused capillaries is variable (34). The functional condition of the capillary bed can be determined by measuring surrogate parameters like skin temperature, blood flow, the movement of blood cells and oxygen supply. The capillaries are comprised of an arterial and a venous segment. The apical bow lies between these two segments (15). The mean diameter of the arterial segment is $11 \pm 3 \mu m$, and that of the venous segment $12 \pm 3 \mu m$ (Fig. 3) (68). These diameters represent the diameters of erythrocyte columns and not those of the actual diameter of the vessels.

Different factors can cause disorders in microcirculation: Firstly, microangiopathies, e.g. in connective tissue diseases or diabetes mellitus. Secondly, macrovascular irregularities, which can cause disorders in the function and in the morphology of the micro vessels, e.g. ischemia or chronic venous insufficiency. Capillary flow depends also on blood viscosity. Furthermore temperature, circadian variation and the effect of drugs are of significance.

The capillaries of the skin can be seen under a microscope only if they are filled with blood (24). Sub papillary plexus can be displayed only in 30% of healthy test persons (46). The expansion and the transparency of the uppermost layer of the skin is the prerequisite of obtaining the best possible evaluation of vessels. The outer layers of the calluses can be made transparent by different technical means, but in some cases when
the skin is much callused, the results are incomplete. Most healthy finger capillaries are typically loop-shaped (15,62) (Fig. 4,22).

**Figure 2** The human blood vessel system (68).
According to data of different authors, 8-17 capillary loops per millimeter can be counted normally; less than 7 is considered pathologic. In healthy subjects the shape and size of each capillary on the same finger of any single person remain constant (15, 55, 80).

Nailfold capillary microscopy is widely used for the diagnosis of Raynaud’s phenomenon (45, 10). In contrast to healthy capillaries (Fig. 4) certain abnormalities are most commonly associated with Raynaud’s phenomenon such as reduced number of capillaries (Fig. 5), avascular fields (Fig. 6), bunch capillaries (Fig. 7), bizarre formation (Fig. 8), torquated (Fig. 9), giant or mega capillaries (Fig. 10) and hemorrhage (Fig. 11).

Figure 3 Normal morphology and density of human nailfold capillaries.
Figure 4 Rarefaction of capillaries.

Figure 5 Avascular Fields.
Figure 6 Bunch capillaries.
**Figure 7** Bizarre capillary formation.

**Figure 8** Torquated capillaries.
Figure 9 Megacapillary.

Figure 10 Hemorrhages*. 

* Indicates additional information or emphasis.
By various control mechanisms the circulation in micro vessels is adopted to the local requisites. Control mechanism influencing the tonus of smooth muscles in the precapillary resistant vessels stem partly from local, and partly from humeral nervous factors (15). Thermal stimuli influence the vessel tonus, i.e. generate vasodilatation when heated and vasoconstriction when exposed to cold. The circulation of finger can be blocked almost completely in the cold from hypothalamus to assist thermoregulation. In the cold, this is caused mainly by a variously sympathetic vasoconstrictor tonus in the arterio-venous anastomoses and on the other side by the rise of local blood viscosity, which is local and conditioned by the cold. The viscosity is dependent on the temperature and between 27 and 37°C there is a linear proportion between the blood temperature and viscosity. In a skin temperature less than 8° and over 45°C the pain fibbers are activated. In the cold vasodilatation can be caused by metabolic factors and axon reflexes in order to protect against frostbites.

Blood flow of the skin capillaries fluctuates considerably under physiological conditions. Rhythmic variation not caused by heart frequency or respiratory frequency occurs. Intraindividual variations of the frequency of blood flow rhythm were found in various areas of skin and demonstrate different local control mechanism (61). Examinations have shown that two different flow types can be discerned (Fig. 12 a-c). One can differentiate between a continuous flow type with more or less distinct variation of velocity, which occurs in the majority of healthy persons (Fig. 12 a) and an intermittent flow type or “on-off” flow type with flow decrease or nearly standstill during 2 to 40 seconds between two velocity peaks, which appears in about 20% of healthy test persons (5,10) (Fig. 12 b, c).
**Figure 11a-c** Laser Doppler registration of blood flow velocity in a finger capillary (CAM 1). Continuous flow type (a) and Intermittent flow types (b, c) (64,65).
In 1919 Basler was the first who measured resting capillary blood cell velocity on the nailfold of a finger (2). Mean capillary flow velocity in healthy people ranges between 0.4 and 0.8 mm/s depending on the measuring technique that was used (Tab.1).

Table 1 Capillary blood flow velocity measured with different techniques (71,72,28)

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>mm/s</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basler</td>
<td>1919</td>
<td>0.6</td>
<td>mechanical</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.11-1.2)</td>
</tr>
<tr>
<td>Bollinger et al.</td>
<td>1974</td>
<td>0.84</td>
<td>frame to frame</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.39-1.74)</td>
</tr>
<tr>
<td>Butti et al.</td>
<td>1975</td>
<td>0.8</td>
<td>frame to frame</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.14-2.36)</td>
</tr>
<tr>
<td>Fagrell/Fronzek</td>
<td>1976</td>
<td>0.64</td>
<td>cross-correlation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.12-2.6)</td>
</tr>
<tr>
<td>Fagrell et al.</td>
<td>1977</td>
<td>0.65</td>
<td>cross-correlation</td>
</tr>
<tr>
<td>Jacobs</td>
<td>1985</td>
<td>0.66</td>
<td>flying spot</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.21-0.98)</td>
</tr>
<tr>
<td>Mahler et al.</td>
<td>1986</td>
<td>0.66</td>
<td>flying spot</td>
</tr>
<tr>
<td>Oestergren/Fagrell</td>
<td>1986</td>
<td>0.67(men)</td>
<td>cross-correlation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.53 (women)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.01-2.8)</td>
</tr>
<tr>
<td>Stuecker et al.</td>
<td>1995</td>
<td>0.47</td>
<td>Laser Doppler</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.14-0.93)</td>
</tr>
<tr>
<td>Present study</td>
<td>2005</td>
<td>0.4</td>
<td>Laser Doppler</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.15-1.13)</td>
</tr>
</tbody>
</table>
II.5 Sildenafil citrate

DESCRIPTION
Sildenafil is a selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5) and is clinically approved to treat erectile dysfunction. Sildenafil, initially known as compound UK-92,480, was initially developed to treat angina pectoris. In phase I clinical trials, the drug turned out to have little effect on angina, but that it could induce marked penile erections.

Sildenafil is designated chemically as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazine-citrate.

Its structural formula is shown in Figure 13.

![Figure 12 Structural formula of sildenafil citrate (52).](image-url)

Sildenafil is a white crystalline powder with a solubility of 3.5 mg/mL in water and a molecular weight of 666.7. Sildenafil citrate is formulated as blue, film-coated rounded-diamond-shaped tablets equivalent to 25 mg, 50 mg and 100 mg for oral administration. In addition to the active ingredient, sildenafil citrate, each tablet contains the following inactive ingredients: microcrystalline cellulose, anhydrous dibasic calcium phosphate, croscarmellose sodium, magnesium stearate, hydroxypropyl methylcellulose, titanium dioxide, lactose, triacetin and FD & C Blue aluminum lake (52).
CLINICAL PHARMACOLOGY

Mechanism of Action

The physiologic mechanism leads to vaso relaxation and involves release of nitric oxide (NO) in the vessels. NO activates the enzyme guanylate cyclase, which results in increased levels of cyclic guanosine monophosphate (cGMP), producing smooth muscle relaxation in the vessel and allowing inflow of blood. Sildenafil has no direct relaxant effect on isolated human vessels, but enhances the effect of nitric oxide (NO) by inhibiting phosphodiesterase type 5 (PDE5), which is responsible for degradation of cGMP in the vessels (Fig.14).

In addition to human corpus cavernosum smooth muscle, PDE5 is also found in lower concentrations in other tissues including platelets, vascular and visceral smooth muscle, and skeletal muscle. The inhibition of PDE5 in these tissues may be the basis for the enhanced platelet antiaggregatory activity of nitric oxide observed in vitro (36,3), an inhibition of platelet thrombus formation in vivo and peripheral arterial-venous dilatation in vivo (33,81,31).
Figure 13 Pharmacodynamic of sildenafil (54).
Pharmacokinetics and Metabolism

Sildenafil is rapidly absorbed after oral administration, with absolute bioavailability of about 40%. Its pharmacokinetics is dose-proportional over the recommended dose range. It is eliminated predominantly by hepatic metabolism (mainly cytochrome P450 3A4) and is converted to an active N-desmethyl metabolite with properties similar to the parent, sildenafil. Both sildenafil and the metabolite have terminal half-lives of about 4 hours.

Mean sildenafil plasma concentrations measured after the administration of a single oral dose of 100 mg to 756 healthy male volunteers is depicted in Figure 15.

![Figure 14](image) Mean sildenafil plasma concentrations in 756 healthy male volunteers (52).

Maximum observed plasma concentrations are reached within 30 to 120 minutes (median 60 minutes) of oral dosing in the fasted state. Sildenafil and its major circulating N-desmethyl metabolite are both approximately 96% bound to plasma proteins. Protein binding is independent of total drug concentrations.
After either oral or intravenous administration, sildenafil is excreted as metabolites predominantly in the feces (approximately 80% of administered oral dose) and to a lesser extent in the urine (approximately 13% of the administered oral dose).

Single oral doses of sildenafil (100 mg) administered to healthy volunteers produced a mean decrease in systolic/diastolic blood pressure of 8.4/5.5 mmHg (52). The decrease in blood pressure was most notable approximately 1-2 hours after dosing, and was not different than placebo at 8 hours (Fig.16). Similar effects on blood pressure were noted with 25 mg, 50 mg and 100 mg of sildenafil, therefore the effects are not related to dose or plasma levels within this dosage range. Larger effects were recorded among patients receiving concomitant nitrates (52).

![Figure 15](image)

**Figure 15** Mean change in systolic blood pressure after administration of sildenafil or placebo in 756 healthy volunteers (52).
CONTRAINDICATIONS

Consistent with its known effects on the nitric oxide/cGMP pathway, sildenafil was shown to potentiate the hypotensive effects of nitrates (52). Sildenafil is contraindicated in:

- Combination with other nitric oxide donors, organic nitrates and nitrates, such as glyceryl trinitrate, sodium nitroprusside, amyl nitrite
- Men for whom sexual intercourse is inadvisable due to cardiovascular risk factors
- Severe hepatic and renal impairment
- Hypotension
- Recent stroke or heart attack
- Hereditary degenerative retinal disorders

ADVERSE REACTIONS

Adverse events reported in placebo-controlled trials in which sildenafil was taken as recommended, on an as-needed basis in flexible-dose are listed in Table 2.
Table 2 Adverse events reported more frequently on drug than placebo by ≥ 2% of patients treated with sildenafil in flexible-dose phase II/III studies (52)

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Percentage of Patients Reporting Event</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SILDENAFIL</td>
</tr>
<tr>
<td></td>
<td>N=734</td>
</tr>
<tr>
<td>Headache</td>
<td>16%</td>
</tr>
<tr>
<td>Flushing</td>
<td>10%</td>
</tr>
<tr>
<td>Dyspepsia</td>
<td>7%</td>
</tr>
<tr>
<td>Nasal Congestion</td>
<td>4%</td>
</tr>
<tr>
<td>Urinary Tract Infection</td>
<td>3%</td>
</tr>
<tr>
<td>Abnormal Vision†</td>
<td>3%</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>3%</td>
</tr>
<tr>
<td>Dizziness</td>
<td>2%</td>
</tr>
<tr>
<td>Rash</td>
<td>2%</td>
</tr>
</tbody>
</table>

† Mild and transient, predominantly color tinge to vision, but also increased sensitivity to light or blurred vision.
Other adverse reactions occurring at a rate of > 2%, but equally common on placebo were respiratory tract infection, back pain, flu syndrome, and arthralgia. In fixed-dose studies, dyspepsia (17%) and abnormal vision (11%) were more common at 100 mg than at lower doses. At doses above the recommended dose range, adverse events were similar to those detailed above but were reported more frequently. Sildenafil was administered to over 3700 patients (aged 19-87 years) during clinical trials worldwide. Over 550 patients were treated for longer than one year. In placebo-controlled clinical studies the discontinuation rate due to adverse events for sildenafil (2.5%) was not significantly different from placebo (2.3%). The adverse events were generally transient and mild to moderate in nature. In trials of all designs adverse events reported by patients receiving sildenafil were generally similar. In fixed-dose studies the incidence of some adverse events increased with dose. The nature of the adverse events in flexible-dose studies, which more closely reflect the recommended dosage regimen, was similar to that for fixed-dose studies.
II.6 Aim of the work

The aim of this work was to evaluate in a prospective double-blinded, placebo-controlled, fixed-dose crossover study the effect of 50 mg sildenafil twice daily versus placebo in patients with severe Raynaud's phenomenon resistant to conventional vasodilatory therapy.

We wanted to answer the following questions:

• What is the normal mean capillary flow velocity detected by laser Doppler anemometry in healthy men?

• What is the normal mean capillary flow velocity in patients with severe Raynaud's phenomenon?

• How does sildenafil influence capillary flow in patients with Raynaud's phenomenon?

• How does sildenafil influence symptoms caused by Raynaud's phenomenon?

• How does sildenafil influence digital ulcerations in patients with Raynaud's phenomenon?

• How is sildenafil tolerated in patients with Raynaud's phenomenon?
III. MATERIAL AND METHODS

III.1 Study subjects

Thirty subjects were recruited for this study. The group of healthy subjects comprised 10 volunteer men (mean age 29 ± 1.2 years), all were physicians or medical students without clinical symptoms, signs of vascular disease and nonsmokers. The group of patients consisted of 20 patients with Raynaud’s phenomenon resistant to vasodilatory therapy. Inclusion criteria were regular occurrence of painful Raynaud attacks (69) and resistance to conventional vasodilatory treatment with at least two agents (74, 79). All patients provided written informed consent, and the study protocol was approved by the institutional ethics committee at the University of Saarland (Saarbrücken, third January 2003). We initially recruited 20 patients and studied 18 of them after 2 patients discontinued use of the study medication because of side effects. Clinical characteristics of the patients are shown in Table 3. The referring rheumatologists diagnosed underlying disease following standard criteria.
Table 3 Clinical characteristics of the patients with Raynaud’s phenomenon treated with sildenafil and placebo

<table>
<thead>
<tr>
<th>Number</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age -- (yr)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>49</td>
</tr>
<tr>
<td>Range</td>
<td>20 - 74</td>
</tr>
<tr>
<td>Female sex -- no. (%)</td>
<td>15 (83)</td>
</tr>
<tr>
<td>Rheumatologic disease</td>
<td></td>
</tr>
<tr>
<td>Systemic Sclerosis -- no. (%)</td>
<td>17 (78)</td>
</tr>
<tr>
<td>Mixed connective-tissue disease -- no. (%)</td>
<td>2 (11)</td>
</tr>
<tr>
<td>No connective-tissue disease – no. (%)</td>
<td>2 (11)</td>
</tr>
<tr>
<td>Therapy before sildenafil</td>
<td></td>
</tr>
<tr>
<td>Nitroglycerine -- no. (%)</td>
<td>7 (39)</td>
</tr>
<tr>
<td>Calcium-channel blockers -- no. (%)</td>
<td>17 (94)</td>
</tr>
<tr>
<td>ACE-inhibitors -- no. (%)</td>
<td>4 (22)</td>
</tr>
<tr>
<td>Intravenous prostaglandin -- no. (%)</td>
<td>3 (17)</td>
</tr>
<tr>
<td>Pentoxifylline -- no. (%)</td>
<td>5 (28)</td>
</tr>
<tr>
<td>Bosentan -- no. (%)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Digital Ulcerations – no. (%)</td>
<td>6 (33)</td>
</tr>
</tbody>
</table>
III.2 Study protocol

Patients were randomly assigned to take placebo or 50 mg of sildenafil twice daily for 4 weeks. After one week of wash-out, patients were switched to the other therapy (Fig. 17).

It was planned prospectively to include 20 patients, to evaluate their mean capillary flow velocity while taking sildenafil or placebo and to compare it to the mean capillary flow velocity of 10 healthy volunteers. All vasodilatory agents were stopped and concomitant medication for treatment of the rheumatic disease remained unchanged during the whole study. The primary outcome variables were frequency and duration of Raynaud attacks and capillary flow detected by nailfold capillary microscopy using a laser Doppler anemometer. Physician’s samples of sildenafil provided by Pfizer Inc. to individual physicians at our institution were collected and the hospital’s pharmacist performed blinding and encapsulation of the tablets. Study started in June 2003 and ended September 2003.

Figure 16 Study design. R denotes Randomisation and NCM Nailfold Capillary Microscopy.
III.3 Evaluation of symptoms in patients with Raynaud’s phenomenon

Symptoms of Raynaud’s phenomenon were assessed by diary cards. When an attack occurred, patients immediately recorded the event and its duration in the diary. At the end of each day patients gave an overall estimation of the last 24 h using a 10 point Raynaud’s Condition Score (0 point = subject felt not handicapped by Raynaud attacks; 10 points = subject felt extremely handicapped) validated as described elsewhere (Figure 18) (48).

Figure 16 Sample page of the Raynaud’s phenomenon attack diary (48).
III.4 Evaluation of capillary flow velocity

Nailfold capillary microscopy was performed and capillary flow velocity was detected using the CAM 1 Laser Anemometer (KK-Technology, Bridleways, Holyford, Devon, England).

All subjects were placed in a sitting position with the hand at heart level, in a quiet, air-conditioned, conveniently tempered room (22 °C) for a period of 30 minutes. Before starting the measurement session in each subject 3 capillaries suitable for flow measurements were identified. Blood cell velocity was measured continuously for 7 min in the same 3 capillaries in each subject at any one time. The skin of the tested hand was heated to a constant level of 37°C by means of an infrared lamp and a controllable heating pad. Skin temperature was controlled by an infrared thermometer. This procedure was chosen to increase capillary flow velocity and to minimize episodes of physiological capillary blood stasis, which may occur frequently and last long in patients with Raynaud's phenomenon.

Healthy subjects were measured on three successive days, thrice a day, at 11h00, 15h00, and 18h00. Due to the higher blood flow velocity in the afternoon (Fig. 32) patients were measured only once between 15h00 and 18h00. The mean capillary blood cell velocity of the patients was assessed as the average of the mean flow velocities in the 3 measured capillaries before the treatment and at the end of each 4-week treatment interval. The dorsal finger phalanx was chosen for all measurements, since the nailfold skin represents one of the nearest possible region having capillaries lying parallel to skin surface. In order to avoid uncontrollable light reflection and at the same time to make the skin transparent, an immersion oil was applied on the part of the skin to be tested (Fig. 19). To prevent the finger from moving, it had to have a stable position. Therefore, the subject was tested in a comfortable sitting position with the hand lying at the same height as the heart, while the arm rested firmly on the pad (Fig. 20) and collateral sustainers stabilized the finger. Using four fiberglass pencils with green-light filter, the skin was illuminated (Fig. 21). After the adjustment of the working distance between the
objective and the surface of the skin, the uppermost capillaries were focused, using the fine-tuning of the microscope under control of the computer display (Fig. 22). After positioning of the laser beam, the velocity in a single capillary was measured (Fig. 23). A clearly audible Doppler signal was used as acoustic control for correct positioning.
Figure 19 Surface preparation.

Figure 20 Positioning of the hand.
**Figure 21** Illumination of the finger.

**Figure 22** Normal morphology of the nailfold capillaries.
Figure 23 Laser Doppler registrations of blood flow velocity in a finger capillary of a healthy subject with physiological blood flow fluctuations.
III.5 Laser Doppler anemometry

The technique of Laser Anemometer was first demonstrated in 1964. Typical applications are studies of velocity and turbulence profiles of liquids and gases in complex systems, such as gas turbines or aerodynamics in wind tunnels. The first in vivo application was the measurement of the velocity of blood cells in an 80 micron diameter retinal artery of an albino rabbit (58), using laser light backscattered at a known angle. Another laser Doppler microscope anemometer (20) was used to measure velocity profiles in arterioles 65-98 microns in diameter. There are several other methods available for measuring blood cell velocity within single capillaries: flying spot, frame to frame, and photometric correlation. They are all image-based techniques and can only measure flows in vessels lying parallel to the surface. Since the measurement is derived from the image, good, high contrast images are necessary and the data is evaluated off-line. Measuring blood cell velocities in vivo using the laser Doppler technique is meanwhile well established and seems to be superior to other methods considering the following aspects. The velocity profiles are displayed on-line under acoustic control. This technique does not depend merely on capillaries lying parallel to the skin surface, but can carry out measurements on any spot with capillaries lying vertical to the skin surface.
The Doppler Effect, named after Austrian physicist J. C. Doppler who first described it for sound in 1842, states that waves emitted from a source moving toward an observer are squeezed; i.e. the wave’s wavelength is decreased and frequency is increased, as shown in Figure 24. Conversely, waves emitted from a source moving away from an observer are stretched; i.e. the wave’s wavelength is increased and frequency is decreased. The waves can be acoustic waves, electro-magnetic radiation or light amplified by stimulated emission of radiation (laser).

**Figure 24** Doppler effect (41).
III.5.1 The CAM1 laser anemometer system

CAM1-system is able to measure red blood cell velocities in small vessels, particularly the capillaries of skin, but also the surface capillaries of any other organ. Skin tissue is relatively transparent and the apex of capillaries can be viewed using a microscope with high illumination. For maximum contrast between red blood cells and the surrounding tissue, green light of 525 nm is used. A schematic and original picture of the CAM1 is shown in Figure 25 a and 25 b.
Figure 25 CAM 1 System. a Schematic of CAM1 bs= beamsplitter, m= mirror l=lens.
b Photography of CAM 1.
A near infrared (780nm) laser produces a 5x10 mm elliptical spot, with a power of about 1 mW. Laser power at the focal point is typically 0.8 to 1 mW with an elliptical spot of about 5 μm by 10 μm, which is focused by lenses l1 (l1, Fig. 25 a) and objective l2 lens (l2, Fig. 25 a). The position of the generated laser beam is adjusted by a lens (l1, Fig. 25 a) so that the waist of the laser beam is in the object plane of the CCD camera and the photodetector. The position of a first mirror (m1, Fig. 25 a) and a beamsplitter (bs1, Fig. 25 a) is adjusted so that the laser beam is in the centre of the image. A beamsplitter is a plate of glass with a thin coating of silver (usually deposited from silver vapor) with the thickness of the silver coating such that, of light incident at a 45 degree angle, one half is transmitted and one half is reflected. The position is set so that the photodetector is aligned with the image of the laser beam focal point. For maximum contrast between red blood cells and the surrounding tissue, green light of 525 nm is used for illumination for the CCD camera. The beamsplitter (bs1, Fig. 25 a) separates the laser light from the green CCD image and the backscattered laser radiation. Another beamsplitter (bs2, Fig. 25 a) splits further the backscattered laser radiation via a second mirror (m2, Fig. 25 a) onto the detector. When CAM1 is positioned and focused so that the laser beam is on a vertical arterial limb of a capillary loop, a small fraction of the laser light is backscattered by blood cells and collected by a second lens (l2, Fig. 25 a). l2 also collects laser light reflected from the surrounding tissue. Laser light backscattered by a moving blood cell shifts the frequency of the light. The magnitude of the shift depends on the angle of scatter and the angle of the blood cell velocity. Since CAM1 collects the reflected light along the same path as the incident light, the frequency shift will only depend on the relative angle, \( \theta \), between the incident beam and the velocity, \( V \).

The frequency shift will be: 

\[
\delta f = \frac{2nV}{\lambda} \cos \theta
\]

Where \( n \) is the refractive index of the medium (the CAM1 uses a value of 1.33) and \( \lambda \) is the laser wavelength (780 nm). The maximum frequency shift obtainable at any capillary blood cell velocity is when \( \theta \) is 0 or 180°.

Of course this cosine dependency exists for all methods using the Doppler effect and for conventional capillary microscopic imaging techniques as well, such as frame to frame and video correlation techniques. In practice, one adjusts the laser beam position for the
strongest signal. This will occur when there are a maximum number of moving blood cells present in the sample volume. In small capillaries, a perpendicular section will provide the maximum number of blood cells in the focal point, since the laser beam has a greater depth of focus than its diameter. Therefore the use of a perpendicular amplifies the signal.

The CAM-1 Laser anemometer system computes mean maximum, minimum and average flow rate in Doppler flow recordings of up to 20 min. Incorrect positioning of the laser beam caused by accidental patient movements were indicated by a fading sound of the Doppler shift and were online marked on the recording. Begin and end of the corresponding time intervals were later offline precisely assigned and were not considered in the calculation of flow velocity.
III.5.2 Electronic data processing

A schematic of electronic data processing is shown in Figure 26. Output from the photodetector is amplified and then bandwidth limited to 68 Hz to 50 kHz, which corresponds to a velocity range of 0.02 to 14.6 mms/1. An analogue to digital converter (ADC) on an interface card in a personal computer digitizes the signal for processing by the CAM1 software. The ADC samples the signal with 12 bit resolution. The basic bandwidth into the ADC is fixed, only the ADC sample rate is changed. The bandwidth is therefore determined by the ADC sample rate. The software allows the Doppler bandwidth to be set to 6.25, 12.5, 25, or 50 kHz, corresponding to an ADC sample rate of 12.5, 25, 50, and 100 kHz, and velocity full scale ranges of 1.8, 3.7, 7.3, and 14.6 mm/s.

Signal, anti-aliasing filters are not necessary. Data is captured in 512 x 12 bit sample blocks. The interval between blocks can be adjusted but is set by default to 20 blocks per second. The maximum rate is determined by the processing power of the computer, although it is not possible faster than 24.4 Hz at the lowest bandwidth setting (6.25 kHz) due to the time required to acquire the 512 samples. A radix-4 fast Fourier transformation (FFT) is performed on each block of 512 samples. When squared this produces a power spectrum with 256 points. On the lowest bandwidth this gives a resolution of 0.0072 mm/s, and 0.057 mm/s on the highest bandwidth. Therefore is CAM1 able to measure blood cell velocities from 0.06 mm/s to 14 mm/s in single capillaries.
III.6 Statistics

The CAM-1 Laser anemometer system computes mean maximum, minimum and average flow rate in Doppler flow recordings of up to 20 min. Using Microsoft Excel and Stat View for Macintosh the data were processed further.

Mean values of Doppler flow were calculated as the average of mean blood cell velocity in the three measured capillaries. The symptom variables were calculated as mean of the cumulative number and duration of Raynaud attacks and daily Raynaud’s Condition Score during each four week study interval. Data are shown as mean values ± standard error. Values under sildenafil treatment were compared with those under placebo by Wilcoxon Signed Rank test. The same test was used to compare the mean capillary flow velocity in healthy volunteers at different times of day. The two-tailed Mann-Whitney U-test was used to compare the capillary flow velocity in healthy subjects and in patients with Raynaud’s phenomenon. A p-value less than 0.05 was considered significant.
IV. RESULTS

IV.1 Sildenafil effect on symptoms in patients with Raynaud’s phenomenon

Figure 27 shows the mean number and cumulative duration of Raynaud attacks in each 4 week treatment period and the mean daily Raynaud’s Condition Score during treatment with placebo and sildenafil.

Figure 27 Symptoms of the study patients while taking sildenafil and placebo.
The frequency of Raynaud attacks was significantly lower (32 ± 13 vs. 48 ± 16, p=0.0049), and the cumulative attack duration significantly shorter (527 ± 123 vs. 1023 ± 227 min, p= 0.0036), while patients were taking sildenafil. Accordingly the Raynaud’s Condition Score during placebo treatment was significantly higher (2.8 ± 0.5 vs. 2.0 ± 0.3 mm/s, p=0.0002). Treatment effect on clinical symptoms was comparable in patients who received sildenafil first and in those who received placebo first (p= 0.2 for the mean frequency of Raynaud attacks, p= 0.16 for the cumulative attack duration, and p= 0.19 for the percentage of change in Raynaud’s Condition Score). Six patients suffered from digital ulcerations. In all of them ulcerations began markedly to heal during treatment with sildenafil (Figure 28). In two patients ulcerations completely disappeared. Ulcerations reappeared or progressed again after treatment with sildenafil was stopped. By contrast, healing of ulcerations did not occur during treatment with placebo.
Figure 28 Examples of ulcerations before (left) and after 4 weeks of treatment with sildenafil (right).
IV.2 Sildenafil effect on capillary flow velocity in patients with Raynaud’s phenomenon

Figure 29 shows nailfold capillaries in a patient with Sharp-Syndrome and a typical original recording of laser Doppler detected flow velocity after treatment with placebo and sildenafil in the same capillary.

Figure 29 Nailfold capillaries in 250-fold magnification (a): + indicates focus of laser Doppler. Laser Doppler flow after treatment with placebo (b) and sildenafil (c) in a capillary of normal shape and width (not shown) in the same patient.
Capillary blood flow after treatment with sildenafil was significantly higher in each individual patient independently of the randomization order (Figure 30 a, b). In patients treated with placebo first, capillary flow velocity did not increase while treated with placebo (0.22 ± 0.04 vs. to 0.14 ± 0.03 mm/s) but did increase significantly after administration of sildenafil (0.66 ± 0.14, p=0.0051) (Fig. 30 a). In patients treated with sildenafil first, capillary flow velocity increased from (0.15 ± 0.03 to 0.44 ± 0.09, p=0.0117) and declined to the baseline level again after administration of placebo (0.15 ± 0.04, p=0.0117) (Figure 30 b). Treatment effect on mean capillary flow velocity was comparable in patients who received sildenafil first and in those who received placebo first (p= 0.37). In the 2 patients with primary Raynaud’s phenomenon, mean capillary flow velocity improved from 0.2 ± 0.1 mm/s (placebo) to 0.9 ± 0.4 mm/s (sildenafil). Altogether mean capillary blood flow increased during sildenafil treatment from (0.14 ± 0.02 mm/s to 0.56 ± 0.01, p=0.0002) by exactly 400 % (Figure 30 c).
Figure 30: Individual laser Doppler flow (average of mean flow rate in 3 capillaries during 7 min in each patient) at base line, after 4 weeks of treatment with placebo and sildenafil in patients treated with placebo first (a) or sildenafil first (b) and mean capillary flow in all patients (c).
IV.3 Adverse effects of sildenafil

One of 20 patients initially included into the study stopped taking sildenafil because of treatment-related headache. Another patient discontinued treatment because of muscle pain in the legs. Later this patient asked for open label treatment with sildenafil because of symptom relapse and reported no more adverse effects. Because of the small sample size both patients were not considered in the analysis of symptoms and capillary flow as an intention to treat. One patient reported transient swelling of the nasal mucosa, 3 patients complained mild, transient headache, 3 patients reported transient facial sensation of heat and 2 patients reported mild nausea after taking the first capsules of sildenafil and another patient reported transient dizziness. There was no significant effect of sildenafil on blood pressure and heart rate (Fig. 31).

**Figure 31** Blood pressure (BP) and heart rate during treatment with placebo and sildenafil in the study patients. bpm indicates beats per minute.
IV.4 Capillary flow velocity in healthy subjects

Figure 32 displays mean capillary flow velocity in healthy volunteers (mean age $29 \pm 1.2$, 26 – 40 years) split by time of day. Mean capillary flow velocity was significantly higher in the afternoon.

![Graph showing capillary blood flow velocity at different times of day.](image)

**Figure 32** Capillary blood flow velocity in healthy volunteers at different times of day.
Figure 33 shows the mean capillary flow velocities at 15h00 in each healthy individual. These values were used for comparison to the flow velocity measurements in patients with Raynaud's phenomenon, which were performed between 16h00 and 18h00. Figure 33 reveals a considerable interindividual variation of capillary flow velocity despite the relatively extended interval of 21 minutes cumulative measurement time. The instantaneous flow velocity varied between 0.15 and 1.13 mm/s.

![Figure 33](image)

**Figure 33** Mean average capillary flow velocity in 10 healthy volunteers acquired at 15h00.
IV.5 Comparison of capillary flow velocity in healthy subjects and patients with Raynaud’s phenomenon

Figure 34 displays the mean capillary blood flow velocity in healthy subjects detected at 15h00 and mean capillary flow velocity in patients with Raynaud’s phenomenon measured at same time of day (16h00 – 18h00) at baseline and after treatment with sildenafil. Mean capillary flow velocity in patients with Raynaud’s phenomenon at baseline was significantly lower as in normal subjects. After treatment with sildenafil mean capillary flow in healthy subjects and patients with Raynaud’s phenomenon did no more differ significantly.

Figure 34 Mean capillary flow velocity in healthy subjects and patients with Raynaud’s phenomenon at baseline and after treatment with sildenafil.
V. DISCUSSION

Clinical context

Vasodilatory therapy of Raynaud’s phenomenon represents a difficult clinical problem because treatment often remains inefficient and may be not tolerated because of side effects. In order to find a new and effective therapy regime for patients with Raynaud’s phenomenon, 18 patients with symptomatic Raynaud’s phenomenon resistant to vasodilatory therapy were treated in a double-blind crossover study with 50 mg sildenafil or placebo twice daily for four weeks. We used diary cards, including a 10-point Raynaud’s Condition Score to evaluate symptoms and measured capillary flow velocity in digital nailfold capillaries with a laser Doppler anemometer. Healing of digital ulcerations after administration of sildenafil was documented photographically. In order to have normal values of physiological capillary blood flow 10 healthy young men were studied. Our results confirm recent case reports on successful occasional use of sildenafil in patients with Raynaud’s phenomenon (59,42)

Laser Doppler anemometry

The condition of the skin depends strongly on the nutritional blood flow. Microcirculation of the skin may be severely altered by Raynaud’s phenomenon. Several techniques have been developed to determine the flow velocity in nailfold capillaries. Quantification of capillary flow velocity by means of the conventional measuring of short-term videotape sequences (flying spot, frame to frame, photometric correlation) has been problematic, since analysis of flow velocity is performed offline and cannot repeated exactly relocating the same capillary volume. Laser Doppler capillary anemometry, however, allows online registration of capillary flow under visual and acoustic supervision in any single capillary over a defined period of time up to 20 min.
The laser Doppler anemometer (CAM1) used in this study provided precisely localized measurements in defined vessels and exact relocation of defined capillaries for consecutive measurements. Thus comparability of measurements in the same patient at different times is warranted. The visual and acoustic control of the position of the laser beam allowed identification of time intervals with incorrectly positioned laser beam. Those intervals were marked online, later precisely assigned offline and were not considered in the calculation of flow velocity.

**Capillary flow velocity in healthy volunteers and patients with Raynaud’s phenomenon at baseline.**

The mean capillary flow velocity in healthy subjects was $0.402 \pm 0.018$ mm/s. This mean resting blood flow velocity corresponds to normal values, which were obtained in other studies using various methods (Tbl.1). The instantaneous capillary flow velocity fluctuated between 0.15 and 1.13 mm/s and the mean capillary flow velocity varied between 0.29 and 0.52 mm/s (Fig. 33). This considerable variability of the nailfold blood flow velocity was shown previously (5,14,21,22,23,27,51,64). Even in adjacent capillaries, measured simultaneously, an asynchronous flow manner with differences up to 0.5 mm/s has been discerned (24). The cause of this phenomenon has not been clarified. Our measurements of capillary flow velocity in healthy subjects showed circadian variation with increase of the blood flow velocity in the afternoon and evening (Fig. 32). The skin is integrated in circadian thermoregulation: in early morning the vessels constrict in order to warm up the body. In the evening, the blood flow increases to emit warmth in order to prepare the body for rest (34). In order to warrant the comparability between measurements in healthy volunteers and patients with Raynaud’s phenomenon we tested all subjects at the same time of day. The mean baseline capillary flow velocity in patients with Raynaud’s phenomenon was $0.14 \pm 0.02$ mm/s. This flow velocity differed significantly from physiological flow in healthy subjects ($p=0.0001$) pointing out the severe impairment of microcirculation in patients with Raynaud’s phenomenon.
Capillary flow velocity in healthy volunteers and patients with Raynaud’s phenomenon after treatment with sildenafil

The mean capillary flow velocity in patients with Raynaud’s phenomenon after treatment with sildenafil was 0.56 ± 0.01 mm/s. In comparison to healthy subjects this flow was no more statistically different (p=0.7372, Fig. 34). Following the results of laser Doppler anemometry the disturbed microcirculation in patients with Raynaud’s phenomenon not only improved but also normalized after treatment with sildenafil.

Sildenafil effect on symptoms in patients with Raynaud’s phenomenon

The frequency of Raynaud attacks was significantly lower and the cumulative attack duration significantly shorter while patients were taking sildenafil (frequency of Raynaud attacks: 32 ± 13 vs. 48 ± 16, p= 0.0049; cumulative attack duration: 527 ± 123 vs. 1023 ± 227 min, p= 0.0036). Accordingly the Raynaud’s Condition Score during placebo treatment was significantly higher (2.8 ± 0.5 vs. 2.0 ± 0.3 mm/s, p=0.0002). Treatment effect on clinical symptoms was comparable in patients who received sildenafil first and in those who received placebo first (p= 0.2 for the mean frequency of Raynaud attacks, p= 0.16 for the cumulative attack duration, and p= 0.19 for the percentage of change in Raynaud’s Condition Score). In all 6 patients with secondary Raynaud’s phenomenon who had chronic digital ulcerations, trophic lesions began to heal clearly and visibly during treatment with sildenafil (Fig. 28). In two patients, ulcerations completely disappeared. Ulcerations reappeared or progressed again after treatment with sildenafil was stopped. By contrast, healing of ulceration did not occur during treatment with placebo. These consistent differences between clinical symptoms of Raynaud’s phenomenon during treatment with sildenafil and placebo clearly indicates the pharmacological effect of PDE-inhibition and might not be explained by chance. Although only patients with severe Raynaud’s phenomenon were included in the study, symptoms remained overall relatively low. This is explained by the fact that the study was performed during summer 2003, which was characterized by a stable and extraordinary warm climate in middle Europe. One might object that symptom scores
during therapy with sildenafil and placebo may not be comparable without temperature and activity control. However, such control cannot be performed in everyday life. Considering the stable meteorological conditions during the study period, it is very unlikely that significant improvement of Raynaud’s Condition Score was caused by differences in ambient temperature. The fact that all patients correctly identified whether they were on treatment with sildenafil or placebo certainly limited blinding of the study and might have influenced patient statements in their daily questionnaires. This might also be the reason why no side effects were reported during placebo treatment. However, corresponding improvement of capillary flow velocity has been objectified by blood flow measurements and partial healing of digital ulcers.

**Sildenafil effect on capillary flow velocity in patients with Raynaud’s phenomenon**

Capillary blood flow velocity was significantly higher after treatment with sildenafil in each individual patient independent of the randomization order, and mean capillary flow velocity increased significantly after treatment with sildenafil but not with placebo. Altogether, mean capillary blood flow velocity increased by more than 400% during sildenafil treatment. Treatment effect on mean capillary flow velocity was comparable in patients who received sildenafil first and in those who received placebo first (p=0.37).

Raynaud’s phenomenon occurs as the result of vasoconstriction of the digital arteries, precapillary arterioles, and cutaneous arteriovenous shunts, causing digital ischemia. Nitric oxide (NO) derived from the vascular endothelium plays an important role in the regulation of peripheral vasomotor tone (77) and has been shown to be involved in initiation and maintenance of penile erection (53) NO activates guanylate cyclase, which results in an increase of cGMP. cGMP is hydrolyzed by phosphodiesterases (PDEs), in particular by the cGMP-specific PDE-5 isoenzyme. Sildenafil is a highly selective and potent inhibitor of PDE-5, that elevates cGMP, resulting in enhanced vasorelaxation. Furthermore, the NO/cGMP pathway resists $\alpha$-mediated vasoconstriction, which may intensify its vasorelaxing properties (73). Sildenafil has been shown to be effective in the treatment for erectile dysfunction (33,43). Its effect is not limited to the corpus
cavernosum, however, as PDE-5 exists in many other tissues. Therefore, sildenafil acts in various different disorders. It reduces, for example, pulmonary and systemic pressure (30,31,81,82), relaxes saphenous veins and pectoral arteries (16), and increases flow-mediated forearm circulation in patients with chronic heart failure (38), type 2 diabetes (17), and coronary artery disease (36). The effects of sildenafil on microvascular disorders such as Raynaud’s phenomenon have not been subject of controlled studies. In the present study, capillary blood flow was severely impaired and sometimes hardly detectable in patients with Raynaud’s phenomenon. Sildenafil led to a 400% increase of flow velocity. As capillaries have no smooth muscle cells, capillary flow velocity depends mainly on the vasomotor tone in the arterioles. Relaxation of the arterioles results in better capillary filling pressure and higher capillary blood cell velocity. Besides these effects on vascular function, sildenafil-associated inhibition of platelet activation, as reported recently (3,36), could have contributed to the improvement of microcirculation and symptoms of Raynaud’s phenomenon. Furthermore, central effects, for example, improvement of cardiac output and/or oxygenation, might have played a role. One might argue the short half-life of sildenafil (about 4 hours) would limit its clinical use. Our data on capillary blood flow velocity, however, as well as the marked reduction of symptoms, are in favor of prolonged functional effects exceeding the plasma half-life of sildenafil. This is supported by recent findings that demonstrate improvement of flow-mediated brachial artery dilation 24 hours after the last dose of sildenafil (17). After statements of our patients, they perceived the impact on digital microcirculation continued about 2 weeks after sildenafil treatment was been stopped. Nevertheless, treatment effect on clinical symptoms and capillary flow velocity was not dependent on randomization order, indicating that the washout period was sufficiently long.
Tolerability of sildenafil in patients with Raynaud’s phenomenon

One can assume that sildenafil may be well tolerated in most patients with Raynaud’s phenomenon. In this study, only 2 patients interrupted therapy with sildenafil because of treatment-related symptoms, and one of them restarted therapy later without side effects. There was also no significant effect of sildenafil on blood pressure (systolic bp: 117.2 vs. 116.7 mmHg; diastolic bp: 72.2 vs. 67.5 mmHg) or heart rate (71.1 vs. 70.9 bpm, Fig. 31).

Clinical significance of the study results

Overall, our results demonstrate for the first time in a randomized study the efficacy of treatment with sildenafil on microcirculation and symptoms in patients with therapy-resistant Raynaud’s phenomenon. PDE-5 inhibition appears to be a promising new approach in patients with microcirculatory disorders.
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