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INVESTIGATIVE REPORT

Exercise-induced Sweating in Healthy Subjects as a Model to Predict a Drug's Sweat-reducing Properties in Hyperhidrosis: a Prospective, Placebo-controlled, Double-blind Study

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The aim of this study was to develop a model to evaluate the efficacy of drugs with expected sweat-reducing properties in healthy subjects in order to select candidate drugs for the systemic treatment of primary generalized hyperhidrosis. A randomized, double-blind, placebo-controlled cross-over study was performed in 8 healthy subjects. Sweating was induced by exercise. The degree of sweating at different exercise levels was determined by measurement of transepidermal water loss. Either the anticholinergic drug oxybutynin or placebo was given before measurements started. No statistically significant differences in transepidermal water loss between active treatment and placebo were found at the different exercise levels. This is noteworthy, as oxybutynin has been proven successful in patients with generalized hyperhidrosis. Thus, the present model does not mimic the situation in patients with primary generalized hyperhidrosis. This may be because this form of hyperhidrosis is not caused only by sympathetic overactivity, as described in the literature, but is based on more complex mechanisms. Further investigations are required fully to understand the pathophysiology of primary generalized hyperhidrosis in order to develop effective human test models. ***Key words: primary hyperhidrosis; model; systemic treatment; pathophysiology; oxybutynin.***

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The human body has an estimated 4 million sweat glands, of which approximately 3 million are eccrine sweat glands. Apocrine sweat glands are located in limited areas. The ratio of apocrine to eccrine glands is 1:1 in the axillae and 1:10 elsewhere. Both eccrine and apocrine glands are innervated by post-ganglionic sympathetic fibres. For eccrine sweat glands, the major neurotransmitter is acetylcholine, and for apocrine glands, catecholamines are the major neurotransmitters. The thermoregulatory centre in the hypothalamus controls body temperature by regulating eccrine sweat output and blood flow to the skin. This centre responds not only to changes in core body temperature, but also to hormones, endogenous pyrogens, physical activity and emotions (1).

Hyperhidrosis is a disorder of excessive sweat production beyond the degree needed to cool down an elevated body temperature (2). Hyperhidrosis can be defined as primary when it occurs in isolation, or secondary when it occurs because of another disease process or treatment. The symptom affects the palms, axillae, plantar surfaces, face, neck and torso in decreasing frequency, but also in different combinations (3). Localized forms can be distinguished from generalized ones in which the entire skin is affected. The aetiology of primary hyperhidrosis is unknown, but it has been associated with increased activity of the sympathetic nervous system (4).

When excessive sweating is not caused by a primary disease or drugs, only symptomatic treatment is possible. A number of treatment options are available for the control of hyperhidrosis. These treatments vary in their invasiveness, efficacy and tolerability. Most treatments are directed locally (non-surgical or surgical), reflecting the mostly local pattern of symptom presentation. Local treatment includes application of aluminium chloride, water iontophoresis and botulinum toxin A. Surgical treatments include thoracic sympathectomy, in which the nerve tracts and ganglia (T2–T3) that transmit the signals to the sweat glands are interrupted, and the removal of axillary sweat glands by excision or curettage (5).

In cases in which the disorder is not local but affects the whole body surface, systemic therapy would be more appropriate. As eccrine sweat glands are primarily stimulated by centres in the hypothalamus using cholinergic sympathetic fibres, oral anticholinergic drugs are a logical choice of systemic treatment.

Clinical studies evaluating systemic treatment of hyperhidrosis are rare. Only a few case reports are available (6, 7).

In the present study we aimed to develop a model in healthy subjects to evaluate the efficacy of drugs with expected sweat-reducing properties. The aim was to design this model in such a way that the results could be extrapolated to patients with generalized hyperhidrosis

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in order to select candidate drugs that might be effective in the treatment of this form of hyperhidrosis.

In this study we used the anticholinergic drug oxybutynin. In the Netherlands, oxybutynin is registered for treatment of pollakisuria and hyper-reflectory urine bladder due to its antispasmodic and anticholinergic actions, with a maximum dosage of 20 mg daily. Oxybutynin predominantly inhibits the muscarine-3 (M3)-receptor, which is present in relatively high concentrations in the eccrine sweat glands (8). Based on the results of a large case series from our hyperhidrotic patient population, we concluded that generalized hyperhidrosis can be treated successfully with orally administered oxybutynin (9).

In order accurately to monitor the effect of oxybutynin, measurements of transepidermal water loss (TEWL) were performed. The theoretical principle of TEWL measurement is based on the fact that it measures passive diffusion of water vapour through the skin. This diffusion flow can be expressed in terms of vapour pressure gradient (10). In the absence of sweating, the value measured indicates the amount of water vapour that passes the stratum corneum by passive diffusion. Determination of sweat gland activity is one of the established applications of TEWL measurement (10). In case of sweating the sweat glands produce massive amounts of water, which evaporates. By the same principle, this diffusion of water vapour by sweating can be quantified by measuring TEWL. Other quantitative techniques for determining the degree of sweating are gravimetric weighing of the amount of sweat collected on absorbent paper in a certain time period (11), and digital image analysis of ninhydrin-stained sheets (12). To our knowledge, there is no literature in which these various techniques are compared with respect to reproducibility and other measurement performances. Given the high sensitivity of TEWL measurement in determining the amount of passive diffusion, this technique may be considered highly suitable for quantitatively measuring the degree of sweating. The accuracy of this technique is high, although there is underestimation at evaporation rates above 80 g/m^{2}h (10).

METHODS

Participants

Ethics committee approval was obtained and all participants received oral and written information and gave their written informed consent before enrolment.

Eight healthy volunteers, mean age 35.1 years (range 22–50 years) were recruited. All subjects were healthy as judged by medical history and were not allowed to have a history of urinary obstruction, prostate hypertrophy, any gastro-intestinal disorders, hiatus hernia, myasthenia gravis, hyper- or hypotension, renal or liver failure, glaucoma, hyperthyroidism, heart disease, smoking, or the use of any medication that might affect the activity of oxybutynin.

Study design and treatment

The study was designed as a randomized, double-blind, placebo-controlled, cross-over study in which participants acted as their own controls. We planned for the participants to receive periods of treatment with oxybutynin and placebo in randomized order, with a washout period of at least 7 days between treatment periods. All participants were asked to refrain from alcohol and caffeine-containing beverages and food for 12 h prior to the study.

Both active treatment and placebo were administered in identical containers. Oxybutynin (Centrafarm, Etten-Leur, The Netherlands) was administered as 5 mg tablets. The investigators did not have access to the randomization code. Blinding was maintained until the data analysis was completed.

Two to 7 days before the first visit (visit A), each participant performed a test exercise in order to determine their individual threshold of sweating. The test was performed on a cycle ergometer (Rehcor, Lode, Groningen, The Netherlands), the minimal resistance was 60 Watt. Every 3 min the exercise level was increased by 30 Watt until sweating occurred. The degree of sweating was determined by measuring the TEWL. Before exercise and after every 3-min period of exercise, measurements were performed. At the level at which sweating occurred (defined as TEWL-value $\geq$ 50 g/m^{2}h, measured with a probe on the central part of the left forearm), exercise was continued for 6 min and measurements took place after 3 and 6 min.

Measurements took place simultaneously on the central part of the left forearm (probe 1) and the central part of the palm of the left hand (probe 2) by the use of a double-probe Tewameter® (TM 300, Courage & Khazaka, Cologne, Germany). The Guidelines of the European Contact Dermatitis Society were followed (13). Each measurement had a minimal duration of 2 min. A measurement was considered stable when the standard deviation (SD) had a level below 0.15, and the graphic display on the main unit showed a horizontal straight curve (14). At that point, the average value (adjusted to 20 s) was read from the display, and noted in a case record form.

Apart from these measurements, the following parameters were evaluated: core temperature (M300A, First Temp Genius, Crawley, Sussex, UK) in the auditory channel, skin temperature (M300A, First Temp Genius, modified for measuring skin temperature through switching mode on "surface") on the left forearm, heart rate and blood pressure.

On the day before visit A and B oxybutynin 5 mg or placebo tablet was taken 3 times a day with an interval of approximately 8 h. On the morning of the test a fourth tablet was taken, 2 h before the measurements started. At the visits A and B the same procedure as during the test exercise was followed. The measurements at visits A and B (interval 1–2 weeks) were stopped at the level at which during the test exercise the individual sweating threshold was reached.

All measurements were performed in an air-conditioned room (21 ± 2^{o}C) and all participants were allowed to acclimatize to this temperature for at least 10 min prior to the cycle test.

Prior to the cycle test on visits A and B the subjects' global impression of sweat inhibition during treatment was scored by a 100 mm visual analogue scale (VAS). The global impression of sweat inhibition was rated running from "Impression of no sweat inhibition" (0 mm) to "Impression of total sweat inhibition" (100 mm) via "Impression of 50% sweat inhibition" marked halfway on the VAS (50 mm).

Statistical analysis

The sample size calculation was based on the detection of differences in TEWL values of 40 g/m^{2}h (sweat reduction of

75%) after topical application of scopolamine (15), a SD of 18.7 g/m²h, α of 0.05 and β of 0.80. It was calculated that 8 subjects would be required.

The predefined primary outcome measures in this experiment were TEWL-values at the different levels of exercise and differences between active treatment and placebo.

Data are expressed as mean values ± SD. The 95% confidence intervals (CI) for the differences were calculated.

Data were analysed using the 2-sided paired Student's *t*-test. In case data were not normally distributed, the 2-sided Wilcoxon signed rank test was used.

Secondary outcome measure was the participants' global impression of sweat inhibition during treatment according to a 100 mm VAS.

RESULTS

All enrolled participants ($n = 8$) completed the study protocol. The individual thresholds of sweating and the identification of low, medium and maximum exercise levels, as determined during the test exercise prior to visit, are shown in Table I.

Primary outcome measure

The results of the placebo exercises and the test exercises prior to visits A and B were similar.

In both treatments sweat production augmented in response to increasing exercise.

Fig. 1A shows the degree of the sweat production measured at the central part of the left arm following the 2 treatments. Mean TEWL values and mean differences (95% CI) between treatments are shown in Table II. Measurements at the lowest and medium exercise level and after 3 min at the maximum level showed a slight numerical superiority for oxybutynin, but there was no statistically significant difference between both treatments.

Fig. 1B shows the exercise course of the sweat production measured on the left hand after both treatments. The mean TEWL-values and mean differences are shown in Table II. Oxybutynin caused no sweat inhibition on the palms.

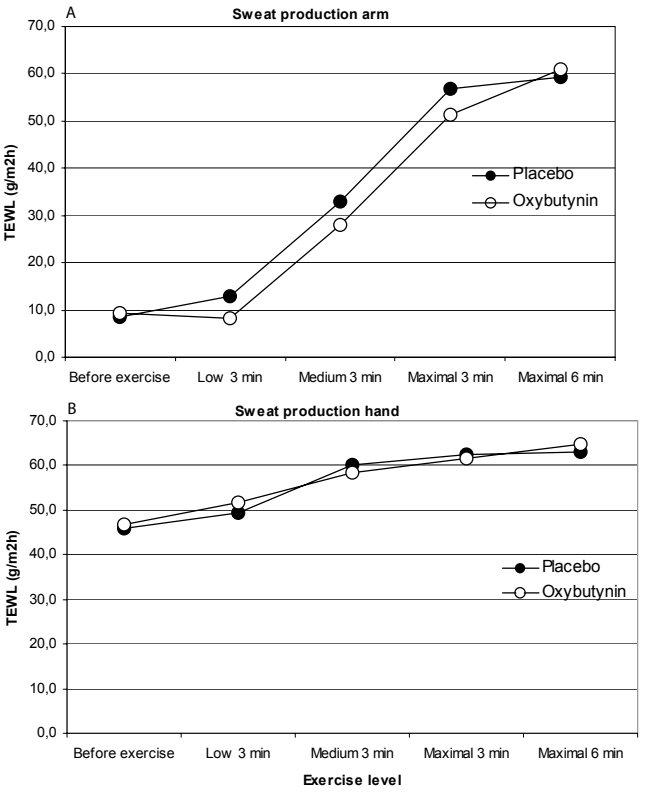

Fig. 1. Sweat production on the arms (A) and hands (B) after different exercise levels expressed as mean transepidermal water loss (TEWL) $n = 8$. Oxybutynin 5 mg or placebo were administered three times on the day prior to the visit and two h before the measurements started.

Table I. *Thresholds of sweating at an individual's low, medium and maximal exercise levels before the treatments*

Subject no.	Sweating threshold (Watt)	Lowest (Watt)	Medium (Watt)	Maximum (Watt)
1	120	60	90	120
2	150	60	90/120	150
3	180	60	120	180
4	180	60	120	180
5	150	60	90/120	150
6	120	60	90	120
7	150	60	90/120	150
8	120	60	90	120

Secondary outcome measures

The majority (7 of 8) of the subjects did not have the impression that sweat production was inhibited after the

Table II. *Transepidermal water loss (TEWL) values and mean differences between treatments after placebo and oxybutynin (n = 8). Data presented as mean (SD) and mean differences (95% CI)*

	TEWL values arm (g/m²h)			TEWL values hand (g/m²h)		
	Oxybutynin	Placebo	Mean difference	Oxybutynin	Placebo	Mean difference
Before exercise	9.4 (8.0)	8.6 (3.9)	0.8 (–4.9, 6.5)	46.8 (14.2)	45.8 (12.1)	1.0 (–10.5, 12.5)
Lowest level 3 min	8.2 (3.2)	13.0 (12.6)	–4.5 (–11.2, 2.1)	51.7 (16.4)	49.3 (14.9)	2.4 (–7.3, 12.1)
Medium level 3 min	28.1 (15.1)	32.8 (21.8)	–4.8 (–14.8, 5.3)	58.5 (12.6)	60.3 (13.7)	–1.8 (–8.7, 5.2)
Maximum level 3 min	51.3 (16.0)	56.9 (11.4)	–5.6 (–16.1, 4.8)	61.5 (15.4)	62.5 (12.3)	–1.0 (–5.1, 3.2)
Maximum level 6 min	61.0 (12.9)	59.4 (8.3)	1.6 (–6.9, 10.1)	64.9 (13.3)	63.1 (11.8)	1.7 (–3.3, 6.8)

SD: standard deviation; 95% CI: confidence intervals.

use of oxybutynin. Only one volunteer experienced a 50% reduction in sweat production according to the VAS.

DISCUSSION

As previous studies showed that generalized autonomic dysfunction in hyperhidrotic patients was caused by overfunctioning of sympathetic fibres (particularly during sympathetic stimulation (4)), we designed a model in which sweating was induced by exercise. During exercise the sympathetic activity increases (and a simultaneous decrease in vagal activity occurs) and therefore would resemble the condition in hyperhidrotic patients as closely as possible.

Treatment of hyperhidrosis with anticholinergic agents aims to inhibit sweat production by blocking the anticholinergic muscarinic receptors at the eccrine sweat glands. In a recent observational study of 14 hyperhidrotic patients treated with oxybutynin (daily doses 7.5 and 15 mg), 11 of 14 experienced improvement in their quality of life, based on their score of the Dermatology Life Quality Index questionnaire, which was administered before and after treatment with oxybutynin (9). However, in the present double-blind, randomized, placebo-controlled study in healthy volunteers we found no evidence for a statistically and clinically significant diminution of sweat production by oxybutynin during exercise.

The discrepancy between the lack of effect of oxybutynin in healthy volunteers and its satisfactory effects in hyperhidrotic subjects is striking. We hypothesize the pathogenesis of primary hyperhidrosis is more complex than described in literature. We formulate 3 mechanisms by which the difference between healthy subjects and hyperhidrotic patients theoretically may be explained.

Primary hyperhidrosis is supposed to be due to hyperperfusion of the frontal area in the cerebral cortex (16). In the current study sweating was induced by exercise, and therefore thermoregulatory circuits are involved. These consist of the anterior pre-optic area in the hypothalamus, the posterior part of the hypothalamus, efferent sympathetic nerve fibres through the medulla oblongata, spinal cord, pre-ganglionic fibres ending in the sympathetic ganglia, post-ganglionic fibres ending at the sweat glands, and the sweat glands themselves (1, 17). Acetylcholine is the neurotransmitter in the pre-ganglionic sympathetic fibres and post-ganglionic sympathetic fibres to the sweat glands (17). Acetylcholine stimulates both nicotinic receptors (located on the synapses between pre- and post-ganglionic fibres) and muscarinic receptors (located on the sweat glands) (17). Oxybutynin exerts antagonism for muscarinic receptors only (8). Apparently, this antagonism is not strong enough to block exercise-induced sweating, in contrast to the spontaneous sweating in hyperhidrosis patients. This may be explained by the hypothesis that the hypothalamus has a sweat centre that controls the palms, soles and axillae, which is under the exclusive control of the cortex, without thermosensitive input (16). A divergence of occupation of nicotinic versus muscarinic receptors in this "cortex-influenced" part and the anterior pre-optic part might account for the above-mentioned discrepancy in action of oxybutynin.

The T2–T3 thoracic ganglia, which are considered to cause sympathetic nerve overactivity, are also in the direct pathway of sympathetic innervation of the heart. Therefore, several studies analysed heart rate variability (HRV) by means of power spectrum analysis in hyperhidrotic patients. HRV has been reported to be a useful tool with a reproducibility sufficient to evaluate both sympathetic and parasympathetic modulation of the cardiovascular system (18, 19).

Power spectrum analysis of HRV performed the day before and after endoscopic trans-thoracic sympathicotomy indicated that hyperhidrotic patients had a sympathetic overactivity, but also a compensatory high parasympathetic activity (20). However, Birner et al. (19) found no evidence of sympathetic dysfunction. Instead they observed parasympathetic dysfunction after autonomic stimulation in hyperhidrotic subjects compared with normal subjects. These results suggest that primary hyperhidrosis is based on a much more complex autonomic dysfunction than generalized sympathetic overactivity and seems to involve the parasympathetic nervous system as well.

Nevertheless, all studies performed investigated primary focal (i.e. palmar, palmoplantar, or axillary) hyperhidrosis, and to our knowledge no data are available on autonomic functions in primary generalized hyperhidrosis.

Furthermore, sweat glands can be stimulated to some extent by adrenaline or noradrenaline circulating in the blood, even though the glands themselves do not have adrenergic innervation (1). This is of special importance during exercise. We hypothesize that, due to the action of (nor)adrenaline, oxybutynin exerts no effect on sweat production during exercise.

We considered measurements on the arm more adequate for use as a target site than those on the palm. TEWL values on the palms are documented to be the highest among all body sites (13). Furthermore, in this exercise study, the outcomes could be biased due to the fact that occlusion of the palms occurred because of holding the handlebars of the cycle ergometer. Finally, there are indications that the palms contain a larger amount of adrenergic sympathetic fibres, which do not respond to anticholinergic stimuli (17), and that this area is more sensitive to psychological influences. All of these factors may explain the very low slope of the dose-response curve on the palm, compared with the forearm.

A model in which sweating in healthy volunteers is induced by exercise may not be suitable to investigate whether primary generalized hyperhidrosis can be treated successfully by an orally administered drug. This might be caused by more complex mechanisms at different physiological levels in the pathology of primary generalized hyperhidrosis.

Further investigations are needed fully to understand the pathophysiology of generalized hyperhidrosis in order to design human test models for selecting candidate drugs for clinical studies in patients with primary generalized hyperhidrosis.

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